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GRUPE FRANCOPHONE D'ÉTUDE DES
PESTICIDES ORGANIQUES D'ORIGINE NATURELLE



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Mercredi 24 septembre

08.30 – Accueil Palais des Congrès

10.00 – Discours officiel - IBMA-PO2N-Qualimediterranée-CCILR

10.40 - Discours officiel – Région-Département-Communauté d'Agglomération

11.00 - Cyril KAO – sous-directeur de la recherche, de l'innovation et des coopérations internationales à la DGER.

11.30 - Jean-Claude MALET – expert référent national « Acquisition de références et usages orphelins » à la DGAL.

12.00 - Xavier LANGLET – expert national agriculture biologique & méthodes alternatives à la DGAL.

12.30 – Déjeuner

SESSION 1 – Botanical and essential oil Insecticide

14.30 – O1 - Conférence plénière- A global view of the development and use of botanical insecticides – Murray B. Isman - Canada

15.10 – O2 – Des composés naturels répulsifs contre l'aleurode Bemisia tabaci – Emilie DELETRE – France

15.30 – O3 – Repulsive, irritant and toxic activities of essential oils against yellow fever mosquito, Aedes aegypti – Thomas MICHEL – France

15.50 – O4 - Évaluation de la toxicité des extraits aqueux de l'algue brune Cystoseira crinita sur les juvéniles de Meloidogyne spp – Dhaouya NEBIH HADJ-SADOK – Algérie

16.10 – Pause

SESSION 2 – Essential oil and antimicrobial activity

16.40 – O5 – Overview and New Development of Bio-Pesticide in China - Guanghui DAI – China

17.00 – O6 – Effectiveness of leaf oil of Pimenta racemosa (Mill) J.W. Moore on R. solanacearum population and bacterial wilt of tomato - Péninna DEBERDT – France

17.20 – O7 – Evaluation in vitro de l'efficacité de 7 huiles essentielles sur Ventura Inaequalis – Caroline DEWEER – France

17.40 – O8 – Search of a natural biocide for the conservation of a wax artwork : chemical and biological screening of essential oils – Vanessa ANDREU – France

18.00 – O9 - Effect of Thymol on olfactory memory and gene expression levels in the brain of the honeybee Apis mellifera - Michel TREILHOU – France

18.30 – Cocktail de bienvenue

Jeudi 25 septembre

SESSION 3 - Elicitor and metabolomic

09.00 – O10 – Conférence plénière - Biocontrol by plant signalling: delivery of low molecular weight natural products using GM, elicitors and other plant – John PICKETT – United Kingdom

09.40 – O11 – The effects of secondary metabolites in carrot on western flower thrips (*Frankliniella occidentalis*) – Rita RAKHMAWATI – The Netherlands

10.00 – O12 – Induction of plant defense and resistance by *Gaultheria procumbens* essential oil, a natural source of methylsalicylate – Bernard DUMAS – France

10.20 – O13 - Crown gall: a new diagnosis method based on the analysis of opines, natural substances produced by infected plants – Floriant BELLVERT – France

10.40 – Pause

SESSION 4 – Botanical Herbicide

11.10 – O14 - Extraction and identification of alkaloids from *Nicotiana glauca* Graham and their phytotoxic effect on *Lactuca sativa* L. germination and seedling growth – Asma RINEZ – Tunisie

11.30 – O15 - Environmental impact of natural β -triketone herbicides - Sana ROMDHANE – France

SESSION 5 – Metabolite and activity of *Bacillus* sp

11.50 – O16 - *Bacillus*-based biological control of plant diseases – Marc ONGENA – Belgique

12.10 – O17 - A novel approach to protect *Bacillus thuringiensis* subsp. *israelensis* spores from the photo inactivation – Patil CHANDRASHEKHAR – Inde

12.30 - Déjeuner

SESSION 6 - Microbial metabolites

14.30 – O18 - Microbial lipopeptides : an emerging family of novel biopesticides – Jacques PHILIPPE – France

14.50 – O19 - Endophyte screening from Indo-spanish medicinal plants: Biotechnological green crop protectants – Azucena GONZALEZ-COLOMA – Espagne

15.10 – O20 - Endosymbiotic actinobacteria from native plants of Algerian Sahara: biological control of the *Rhizoctonia solani* damping-off and tomato plants growth promoting activities – Yacine GOUDJAL – Algérie

15.30 – O21 - Biocontrol properties of Plant Growth-promoting Rhizobacteria – Claire PRIGENT-COMBARET – France

15.50 – O22 - Suivi et impact de *Rhodococcus erythropolis* R138, agent de lutte biologique ciblant les bactéries responsables de la jambe noire sur culture de pomme de terre – Jeremy CIGNA – France

16.10 – Pause poster

17.10 – O23 - Non-ribosomal Peptides from Entomogenous Fungi – HU QUIONGBO – China

17.30 – O24 - Biocontrôle de la pourriture brune du cacaoyer par des *Trichoderma* - Etude des métabolites et de leur détoxification – Didier BUISSON – France

17.50 – O25 - Marine-derived *Trichoderma* strains producing peptaibols as a potential source of new biocontrol agents - Anne-Isaline VAN BOHEMEN – France

18.10 – O26 - Procédés de Fermentation en milieu solide et bioréacteur à usage unique pour la production de biopesticides – Sevastianos ROUSSOS – France

18.30 – Assemblée Générale Groupe PO2N

19.30 - Dégustation de vin et dîner de Gala (Sur réservation)

Vendredi 26 septembre

SESSION 7 – Prospective session : new concept, Screening of news products and results of innovative project.

09.00 – O27 - Conférence plénière - Phéromones et odeurs de plantes: Comment extraire un signal d'un environnement olfactif – Michel RENOU -France

09.40 – O28 - Sustainable plant protection strategy for artichoke downy mildew – Marie TURNER – France

10.00 – O29 - Elicitor screening to protect winter wheat against Septoria tritici Blotch: preliminary results - Geraldine LE MIRE – Belgique

10.20 – O30 - Evaluation de l'efficacité des différents biofertilisants sur les populations résiduelles du puceron noir de la fève Aphis fabae - Wissem CHAICHI – Algérie

10.40 – Pause

11.10 – O31 - Activity and Characterization of hypoiodide and hypothiocyanate ions, two active molecules against plant pathogens – Françoise BAFORT – Belgique

11.30 – O32 - Utilisation de micro-doses de sucres en protection des cultures. Synthèse du projet Usage – Ingrid ARNAUD – France

12.00 – Discours de clôture

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A global view of the development and use of botanical insecticides

Murray B. Isman

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The spectacular success of synthetic chemical insecticides, beginning in the 1940s with the organochlorines and organophosphates, and later with the pyrethroids and neonicotinoids, relegated botanical insecticides – those derived directly from plants – to a trivial share of the crop protection/pest management market for many decades. In spite of this, interest in the discovery of new plant natural products with insecticidal properties has seen a marked increase over the past 30 years, based on analysis of scientific publications on botanical insecticides. For example, in 1980 less than 3% of all papers on insecticides were concerned with botanicals whereas in 2011 more than 20% of insecticide papers published that year dealt with botanicals. However, the translation of theory to practice has been substantially less impressive, with only a handful of new botanical insecticides seeing commercialization in recent years. Between 1980 and 2010 only two new types of botanicals were introduced in the USA, those based on azadirachtin (principally in the form of semi-refined neem seed extracts), and those based on a limited range of plant essential oils. Research interest in plant essential oils as insecticides has exploded worldwide in the past decade, but again commercial success has been far more modest.

Rigorous regulatory schemes in the EU, USA and some other advanced countries have been a significant impediment to commercialization of botanicals, with a few exceptions. In these areas, botanicals are likely to remain specialty products for use in public health, urban pest management and in the production of organic foods. However, introduction of new botanical insecticides is moving at a faster pace in China, Latin America and Africa, arguably regions where humans have suffered some of the worst cases of poisoning and environmental contamination. In some of these jurisdictions, the use of botanicals is far more justifiable, as the source plant species are often locally abundant, accessible and inexpensive. Even crude preparations of certain plants can provide a reasonable level of efficacy with wide margins of safety to users and consumers, and are often more cost effective than synthetic insecticides. Botanical insecticides currently in use and those recently introduced in different jurisdictions will be discussed.



Des composés naturels repulsifs contre l'aleurode *Bemisia tabaci*

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L'utilisation de filets anti-insecte pour protéger les cultures horticoles est un outil efficace et durable surtout contre les lépidoptères. Cependant, si cette barrière physique et visuelle réduit les infestations des petits insectes tel que l'aleurode *Bemisia tabaci*, elle ne les empêche pas de passer à travers les mailles utilisées sous climat tropical. Une solution possible serait de combiner un filet anti-insecte avec un produit répulsif. En laboratoire nous avons testé l'efficacité de filets traités par des composés naturels contre *B. tabaci*. Parmi les 20 huiles essentielles testées, les plus répulsifs ont été l'aframomum (*Aframomum pruinosum*), la citronnelle (*Cymbopogon winterianus*), la cannelle (*Cinnamomum zeylanicum*), le géranium (*Pelargonium graveolens*) et la sariette (*Satureja Montana*). A l'exception de la citronnelle, ces quatre extraits de plantes étaient aussi les plus irritants et les plus toxiques. La composition de ces extraits a été analysée par chromatographie gazeuse. Les composés majoritaires de la cannelle et de la citronnelle ont été testés seuls puis en mélange sur *B. tabaci* en utilisant le même dispositif que pour les extraits de plante. Les propriétés de repulsion, d'irritation ou de toxicité ont varié avec le produit et la concentration et ces deux variables se sont révélées indépendantes. Il est donc possible que les mécanismes d'action de l'irritation, de la répulsion et de la toxicité soient différents. Nous avons observé également des effets additifs, synergiques ou antagonistes des composés bioactifs en mélange ce qui suggère des interactions entre les composés. L'utilisation de composés répulsifs, en combinaison avec un filet pour la protection des cultures horticoles contre les aleurodes est discutée ainsi que l'utilisation de plantes compagnes, qui pourraient produire et relâcher ces composés bioactifs en continue.

Repulsive, irritant and toxic activities of essential oils against yellow fever mosquito, *Aedes aegypti*

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There has been renewed interest in finding a use for natural plant extracts as alternative sources for pesticides since these extracts and their constituent compounds are normally considered safer than chemicals for human health and environment [1]. In this respect, medicinal and aromatic plants from the French Guyana and Martinique (Carribean Island) were evaluated for anti-mosquito activity against *Aedes aegypti*. The essential oils were obtained by hydrodistillation and their chemical profiles were determined using GC/FID and GC-MS. The identification of the separated volatile organic compounds was accomplished through comparison of mass spectra of peaks with those stored in NIST and Wiley libraries. Additional confirmation was achieved by comparison of retention indices from compound peaks. Repulsive, irritant and toxic effects of essential oil were tested at 1 and 0.1% on adult female. Interesting results were found with some essential oils inducing strong toxicity compared to DEET (N,N-Diethyl-meta-toluamide). Furthermore noteworthy knock-down effect, similar to commercial pyrethroid, was also observed. In this case, essential oil exposure at both concentrations induced physiological troubles for mosquitoes.

[1] Isman IB (2006) Annu Rev Entomol 51:45–66



Évaluation de la toxicité des extraits aqueux de l'algue brune *Cystoseira crinita* sur les juvéniles de *Meloidogyne* spp.

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Résumé

Les potentialités nématocides des extraits aqueux de l'algue brune *Cystoseira crinita* ont été testées in vitro sur les larves (L2) de *Meloidogyne* spp. Les résultats ont montrés que la toxicité des extraits varie significativement en fonction de la saison de prélèvement de l'algue, des concentrations des extraits et du temps d'immersion. Les extraits aqueux de *Cystoseira crinita* prélevée en période printanière ont montré une toxicité plus importante vis-à-vis des larves ; 100% de mortalité est enregistrée dès les 24h d'exposition dans l'extrait pur. Par contre, pour les extraits de l'algue récoltée en période estivale, l'effet biocide n'apparaît qu'après 72h d'immersion dans l'extrait pur. Par ailleurs la toxicité diminue dans les extraits dilués au ½ et au ¼.

Mots clés : Extraits Aqueux, *Cystoseira crinita*, *Meloidogyne* spp., Toxicité

Evaluation of the toxicity of aqueous extracts of the brown alga *Cystoseira crinita* on juveniles of *Meloidogyne* spp.

Abstract

Nematicides potential of aqueous extracts of the brown alga *Cystoseira crinita* were tested in vitro on larvae (L2) of *Meloidogyne* spp. The results showed that the toxicity of extracts varies significantly according to the season of harvest seaweed, the concentrations of the extracts and the immersion time. The aqueous extracts of *Cystoseira crinita* collected in spring period showed greater toxicity vis-à-vis the larvae; 100% mortality was recorded after 24 hours of exposure in the pure extract. Whereas extracts of seaweed harvested in the summer, the biocidal effect appears only after 72 hours of immersion in pure extract. In addition, the toxicity decreases in extracts diluted ½ and ¼.

Keywords: Aqueous Extracts, *Cystoseira crinita*, *Meloidogyne* spp., Toxicity.



Overview and New Development of Bio-Pesticide in China

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The development of phytochemical pesticide in China was briefly overviewed in this presentation. A natural substance, D-pinitol, has a control effect on the cucumber powdery mildew (*Sphaerotheca fuliginea*), and its formulation's control effect was confirmed in the field in 4 provinces in China during 2 years test. Its extraction, isolation from the plants and structural identification were presented. The tests of acute toxicity, environment effects, residue in the soil were confirmed, and the mutagenic and subchronic toxicity test results will be available at the beginning of 2015, when it probably has the homologation in the Chinese Agriculture Ministry. This new phytochemical fungicide is actually used in a small scale and its effect on other pathogens is evaluated. To reduce the cost of the phytochemical pesticide so that it will be easier to industrialization, the by-products of some food manufactories, the waste of agriculture were used to try for phytochemical pesticide test, and some interested effects on control of the powdery mildew and downy mildew of cucumber, and *Tetranychus cinnabarinus* in the greenhouse were confirmed.

Effectiveness of leaf oil of *Pimenta racemosa* (Mill) J.W. Moore on *R. solanacearum* population and bacterial wilt of tomato

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Bacterial wilt caused by *Ralstonia solanacearum* is the most widespread plant disease in tropical and subtropical regions. In Martinique (French West Indies), the bacterial wilt situation changed dramatically after 1999 with the emergence of strains of *R. solanacearum* assigned to a new genotype, phylotype IIB/4NPB, which is highly pathogenic for solanaceous crops, especially tomato. Due to the limited efficacy of current strategies for the management of bacterial wilt, new control strategies such as biorational soil treatments are required. We investigated the effects of three differently scented chemotypes of essential oils of *Pimenta racemosa* var. *racemosa* (lemongrass, aniseed and clove scents) on the emerging strain of *R. solanacearum* (phylotype IIB/4NPB), their potential use as biofumigants to reduce bacterial wilt disease of tomato, and their effect on tomato growth. Three concentrations of the oils (0.04, 0.07, and 0.14% vol/vol) were evaluated by *in vitro* culture amendment assays and *in vivo* experiments in a greenhouse. In the culture amendment assays, with lemongrass scented and aniseed scented oil chemotypes at concentrations of 0.04% and 0.07% respectively, both oils significantly reduced the growth of *R. solanacearum* compared with the control, and at 0.14% they completely inhibited bacterial growth. With the clove scented chemotype, complete inhibition of *R. solanacearum* growth was achieved at all the concentrations tested. No incidence of tomato bacterial wilt was observed after treatment with the clove scented chemotype of *Pimenta racemosa* when the tomato plants were planted in essential oil-treated soil. In the untreated control soil, bacterial wilt affected 64% of the tomato plants whereas with the clove scented chemotype at a concentration of 0.14%, no bacterial wilt was observed on tomato. Treatment with the clove scented chemotype significantly increased the growth of tomato plants compared with the untreated control. These results suggest that essential oil of *Pimenta racemosa* could be used as a soil biofumigant in biocontrol-based management strategies for bacterial wilt of tomato.

Evaluation in vitro de l'efficacité de 7 huiles essentielles sur *Venturia inaequalis*

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Porté par l'ITAB et financé par le Ministère de l'Agriculture, le projet CASDAR intitulé « évaluation de l'intérêt d'utiliser des huiles essentielles en protection des cultures » (2013-2015) se propose d'étudier l'efficacité biofongicide in vitro et in planta de plusieurs huiles essentielles sur plusieurs modèles biologiques. Il s'agit ici d'étudier l'efficacité de 7 huiles essentielles sur les spores de *Venturia inaequalis*, le champignon responsable de la tavelure du pommier pouvant causer jusqu'à 70 % de pertes de rendement. Les huiles essentielles sont testées seules ou additionnées d'un adjuvant (Héliosol). Elles sont testées en milieu liquide en microplaques comparativement au cuivre (CuSO_4) et au soufre. La gamme de concentrations employée permet de calculer les CI50 pour chaque modalité grâce à l'aide d'une régression non linéaire. Les expérimentations sont réalisées au moins trois fois de manière indépendantes et permettent de réaliser des tests statistiques de type ANOVA. Parmi les 7 huiles essentielles, les résultats montrent que certaines sont plus efficaces que d'autres. Les huiles essentielles les plus actives le sont également par rapport au cuivre et au soufre. Si toutes les huiles essentielles présentent des efficacités fongicides, l'ajout d'Héliosol ne permet pas cependant d'augmenter cet effet bio-fongicide.

Search of a natural biocide for the conservation of a wax artwork : chemical and biological screening of essential oils

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The « Chambre des certitudes » is an artwork created by the german artist Wolfgang Laib inaugurated in 2000. It's constiting of a cavity inground in the Roc-del-Maure (in the village of Arboussols, Pyrénées-orientales, south of France) whose walls were completely covered with beeswax. This artwork has been subjected for several years to a more and more important degradation by fungal strains. In an issue of restoration and preservation, a research project was initiated in order to i) isolate and identify strains responsible for this degradation ii) find an effective natural control, according to the wishes of the artist.

Two fungal agents causing degradation could be identified by 16S rDNA sequencing. Incriminated species are *Penicillium olsonii* and *Cladosporium cladosporioides*.

A second part of the project consisting in the search of a natural biocide to use in a closed environment to fight against these strains. The choice fell on solutions based on essential oils (from organic farming and locally produced). To date, 20 essential oils were tested in an *in vitro* system developed in CRIOBE and were analyzed by gas chromatography coupled to a mass spectrometer to identify potential active compounds. Several biological screenings were used to select essential oils with encouraging *in vitro* activities. These include essential oils of peppermint (two chemotypes), oregano and common thyme (see Figures 1 and 2). MIC were determined for these four essential oils on the two targets strains.

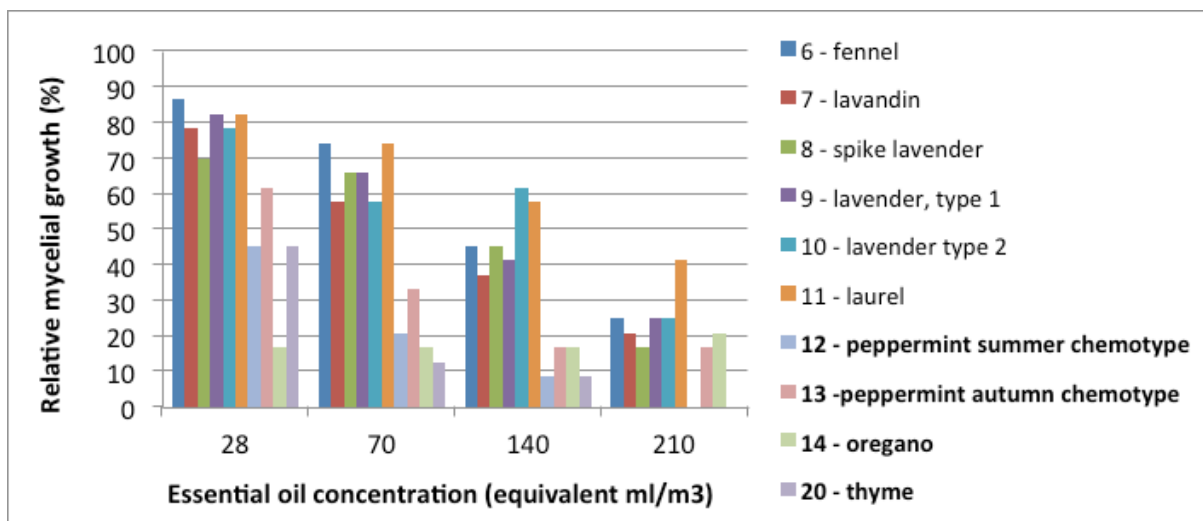


Figure 1: Essential oils effect on *Penicillium olsonii* growth after 4 days incubation

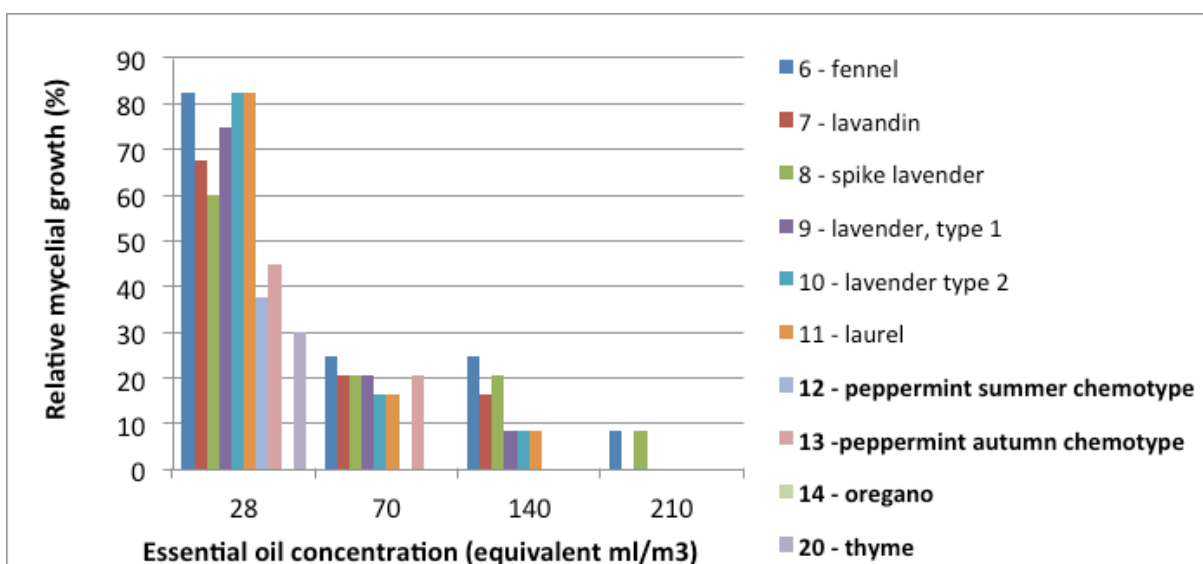


Figure 2: Essential oils effect on *Cladosporium cladosporioides* growth after 4 days incubation

In order to optimize the effectiveness of the treatment on the two targets, assays with essential oils mixtures are in progress, as well as strains culture on synthetic-wax medium to study their behavior on the environment.

In fine, the aim is to provide an essential oils mixture able to limit the development of degrading isolates or even eradicate them. For this, it is planned to test soon selected mixtures *in situ*.

Key words : waxroom – conservation – fungal strains – essential oils - biocide

Effect of Thymol on olfactory memory and gene expression levels in the brain of the honeybee *Apis mellifera*

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The problems of hive weakening and depopulation encountered by beekeepers over the last 10 years have driven the development of much research. A part of this has focused on the fight against the *Varroa destructor* ectoparasite, which is regarded as an important scourge of bees. Initial treatments were based on synthetic pesticides such as coumaphos, fluvalinate, or amitraz. However, the development of resistance caused by some synthetic molecules and/or the undesired transfer to the honey, as well as the increasing demand for organic agricultural products, has lead beekeepers to abandon these active substances in favor of natural products such as essential oils.

Thymol, a phenolic compound from *Thymus vulgaris*, is an effective acaricide, especially concerning the *Varroa* ectoparasite [1], and it has been shown that this monoterpene can kill *Varroa* without noticeable loss of bees [1]. It has been used for several years in the form of evaporating tablets (Apilife Var®) or gel boxes (Apiguard®) that beekeepers can place in hives after honey harvest. Nonetheless, in spite of the success obtained using thymol against *Varroa*, few studies have covered its sublethal effects on bees [1,2]. We present here the results of the “BeeThym” research program where the side effects of thymol on bees’ behaviour have been assessed. Initial studies under laboratory conditions demonstrated an impairment of phototaxis in bees after exposure to sublethal doses of thymol or Apilife Var® [3,5]. In a second part, its effects on olfactory memory were explored plus gene expression in the brain of honeybees from treated hives. The octopamine receptor OA1, Rdl (coding for a GABA receptor subunit) and trpl genes expression were assessed after exposure to sublethal doses. Data indicated that the genes coding for the cellular targets of thymol could be rapidly regulated after exposure [4]. Thymol levels in the brain, the body and the beeswaxes of bees have been monitored throughout the study [5]. However, memory and sensory processes should be investigated in bees after chronic exposure in the hive to thymol-based preparations.

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Biocontrol by plant signalling: delivery of low molecular weight natural products using GM, elicitors and other plant-based technologies

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Plants signal damage to pests and also, positively, to the natural enemies of pests. Small lipophilic natural products from both plants and pests can be delivered using GM (1) and managed using defence-related elicitors in processes involving both aerial (2) and rhizosphere (3) signalling. Delivery of these signal compounds can use companion plants, which also demonstrates the practical value of such approaches (4, 5).

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2. T Bruce *et al.* cis-Jasmone induces Arabidopsis genes that affect the chemical ecology of multitrophic interactions with aphids and their parasitoids. Proceedings of the National Academy of Sciences USA 105, 4553-4558, 2008
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The effects of secondary metabolites in carrot on western flower thrips (*Frankliniella occidentalis*)

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Using an eco-metabolomic approach, comparing the NMR (Nuclear Magnetic Resonance Spectroscopy) profiles of thrips resistant and susceptible varieties, the metabolites sinapic acid, β -alanine and luteolin, have been identified to be associated with host plant resistance to western flower thrips (*Frankliniella occidentalis*) in carrot. All three metabolites caused thrips mortality at plant concentrations (Leiss *et al.*, 2013). In this study, we combined these metabolites to investigate effects on thrips mortality and oviposition. Each metabolite was tested singly, in dual and three way combinations, using 1:1 ratios, at 6 concentrations ranging from 6,25 to 150% of the plant concentration. *In-vitro* thrips mortality and oviposition bioassays were applied. All single metabolites had stronger effects on thrips mortality compared to all combinations. Sinapic acid caused the strongest effect with an LC_{50} of 33%. Only the combination of sinapic acid and alanine at 150% plant concentration (LC_{50} : 28%) indicated some additive effect of compounds on thrips mortality, while the combination of alanine and luteolin indicated some antagonistic effects at plant concentration (LC_{50} : 280%). Independent of mortality, oviposition was significantly inhibited by all single compounds at all concentrations. Besides the mixture of all three compounds at plant concentration, mixtures did not cause a stronger inhibition of oviposition compared to single compounds. Our results demonstrate that sinapic acid, β -alanine and luteolin are more effective against thrips as single compounds rather than as 1:1 mixtures. While thrips mortality was affected at 100% plant concentration, oviposition was already inhibited at 6% of the plant concentration.

Interestingly, sinapic acid and luteolin did not only show a negative effect on thrips, but as anti-oxidants, are also linked to positive human health effects such as prevention of cancer and cardio-vascular diseases. As such these compounds do not only contribute to the development of biopesticides but also to human health improvement. Therefore, our results are of great relevance for practical implementation in sustainable crop protection programmes.

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Induction of plant defense and resistance by *Gaultheria procumbens* essential oil, a natural source of methylsalicylate

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Essential oil from *Gaultheria procumbens* is mainly composed of methylsalicylate (>99%), a compound which can be metabolized in plant tissues to salicylic acid, a phytohormone inducing plant immunity against microbial pathogens. The potential use of *G. procumbens* essential oil as a biocontrol agent was evaluated on the model plant *Arabidopsis thaliana*. Expression of a selection of defense genes was detected 1, 6 and 24 hours after essential oil treatment (0.1 ml/L) using a high-throughput qPCR-based microfluidic technology. Control treatments included methyl jasmonate and a commercialized salicylic acid analog, acibenzolar-S-méthyl (BION). Strong induction of defense markers known to be regulated by the salicylic acid pathway was observed after the treatment with *G. procumbens* essential oil. Treatment induced the accumulation of total salicylic acid in the wild -type *Arabidopsis* line Col-0 and analysis of the *Arabidopsis* line *sid2*, mutated in a salicylic acid biosynthetic gene, revealed that approximately 30% of methylsalicylate sprayed on the leaves penetrated inside plant tissues and was demethylated by endogenous esterases. Induction of plant resistance by *G. procumbens* essential oil was tested following inoculation with a GFP-expressing strain of the *Arabidopsis* fungal pathogen *Colletotrichum higginsianum*. Fluorescence measurement of infected tissues revealed that the treatment led to a strong reduction (70%) of pathogen development and that the efficacy of the *G. procumbens* essential oil was similar to the commercial product BION. Together, these results show that the *G. procubens* essential oil is a natural source of methylsalicylate which can be formulated to develop new biocontrol products.



Crown gall: a new diagnosis method based on the analysis of opines, natural substances produced by infected plants.

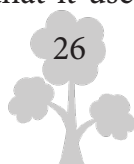
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Crown gall is an economically important phyto-bacteriosis affecting many plants of agronomic and horticultural interest. It is responsible for 10% of losses in nurseries without possibility of efficient curative control and can lead to perennial soil contamination. This plant disease is caused by bacteria of the genus *Agrobacterium* that harbor a Ti plasmid (pTi). A DNA fragment of this plasmid (the T-DNA) is transferred into plant cells and integrated into their nuclear genome. Transformed plant cells produce phytohormones a feature that leads to their proliferation (formation of tumors). Transformed cells also produce specific compounds termed opines. These compounds are trophic resources for pathogenic agrobacteria and some induce the conjugative transfer of the pTi causing the diffusion of the pathogenicity among agrobacteria and extended soil contaminations. Some molecular tools for the detection of agrobacteria and pTi exist, but they are time consuming and delay diagnosis. Moreover, *Agrobacterium*-related infections are sometimes difficult to detect because other plant pathogens unrelated to agrobacteria, can induce similar symptoms on some plants. Furthermore, detection methods are based on a search of bacterial target DNA, which may disappear after plant transformation. In contrast, the production of opines continues in the tumor. Thus, the detection of opines in plants can allow a confident diagnosis of the bacterial origin of tumors. It also provides epidemiological data, as different sequences of T-DNA can induce the production by transformed cells of different sets of opines that are pTi-dependent. Hitherto, the different methods to detect opine require chemical modifications (derivatization, use of reagents for revelation) which did not allow the simultaneous detection of all types of opines and had a low sensitivity. In this study, we developed and validated a direct method for detection, identification and quantification of opines by LC-HRMS and LC-MS/MS in plant's extracts. This method was tested for different plants of interest. This approach will be a relevant tool for the early and fast diagnosis of crown gall. It will be also of interest for epidemiological and ecological studies and, in order to go further, it can be combined with Imaging Mass Spectrometry and biomolecular analyses to understand tumor development and effect of opines on rhizosphere bacterial communities, including interactions with biocontrol agents such as strain K84 that it used to control some cases of crown gall.



Extraction and identification of alkaloids from *Nicotiana glauca* Graham and their phytotoxic effect on *Lactuca sativa* L. germination and seedling growth

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ABSTRACT

In the present study, leaves, flowers, stems and roots of *Nicotiana glauca* Graham were examined for the presence of biologically active alkaloids. Petroleum ether and methanol were used and after extraction, fractions were analysed and alkaloids identification was carried out by gas chromatography-mass spectrometry (GC-MS) and by nuclear magnetic resonance (NMR). The results showed that all methanol fractions containing Anabasine (3-(2-piperidyl) pyridine) and the richness of *N. glauca* different organs is ranked in the following order: stems (95%)> Flowers (82%)>leaves (71%)>roots (19.5%). In addition, scopalamine (19%) and hyoscyamine (13.5%) were identified in roots methanol extract. The fraction with the highest content of anabasine was evaluated on seed germination, water content, root and shoot length of *Lactuca sativa* L. (lettuce) at different concentration (100, 500, 1000, 1500, 3000, 6000 and 9000 ppm). Results revealed that the anabasine was very phytotoxic for all measured parameters. At the higher dose (9000 ppm), an inhibition of 23%, 66.5%, 93.5% and 60% was recorder for germination index, water content root and shoot length. Our findings showed that *N. glauca* can be used as potential source of phytotoxic compound, especially of anabasine.

Key words: Alkaloids, *N. glauca*, Phytotoxic effect, Anabasine, lettuce.



Environmental impact of natural β -triketone herbicides

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Nowadays, more and more concern is shown to the possibility of adverse effects of pesticides on human health and ecosystems. The extended risk assessment and ecotoxicological monitoring of plant protection products, including their main degradation products, is already required within the framework of European legislation. Therefore, recent European laws are converging to reduce the amounts of pesticides used, and thereof, reduce their associated risks. As a consequence, there is a need for new classes of herbicides. The TRICETOX project, a French research project funded by Agence National de la Recherche, aims to study the β -triketone herbicide family, with a focus on four compounds, two synthetic ones, sulcotrione and mesotrione and two natural ones leptospermone and myrigalone. As many natural products, biopesticides are considered as biodegradable and less toxic than synthetic herbicides. However, studies on the fate and effects of the natural products in the soil and water environment are practically non-existing. In this context, TRICETOX project aims to evaluate the possible risks and adverse effects of the two natural β -triketone herbicides on environment by addressing their soil fate and behavior in two different types of soil under laboratory conditions. In a first phase, dissipation time of leptospermone in soil was studied using a microcosmic approach. Our results have demonstrated that half-lives were ranged between 6 and 9 days. This study showed that the dissipation of leptospermone in the soil was essentially influenced by biotic factors. Hydrolysis kinetic under natural conditions showed a stable profile of the molecule. In contrast, the photolytic behavior of leptospermone carried out under drastic conditions was rapid and pH-dependant, with a half-life of 80 min at pH 9. Comparison of these results with those previously obtained with sulcotrione showed that both molecules have approximately the same environmental fate.

Bacillus-based biological control of plant diseases

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Bacilli are among the most important and promising biocontrol agents of microbial origin. These bacteria usually display high ecological competence allowing efficiently colonizing plant tissues and therefore competing for niche and substrate. Moreover, they produce a wide array of antibiotics to directly inhibit phytopathogens and some isolates also retain the potential to stimulate immune responses in the host plant leading to the so-called induced systemic resistance (ISR) phenomenon. We will present the new insights provided by the recent research on molecular aspects of these main mechanisms of biocontrol. Unfortunately, *Bacillus* like other microbial products often provides only partial protection against fungal pathogens and pests. Another weakness is their inconsistent effect. As the active ingredient is a living organism, its efficacy is more strongly dependent on application conditions compared to conventional pesticides. The activity of the beneficial organism depends on its global ecology, on its interactions with the plant host, the pathogen and the biotic and abiotic environmental parameters. Unfortunately, our knowledge about the ecology of most beneficial bacteria used today is still poor, limiting rational field applications. We will illustrate that, in the case of *Bacillus*, one of the main causes of this lack of information is the often-observed deficiency in connections between field trials and more controlled laboratory experiments. For instance, it is often speculated that bioactivity and high occurrence of *B. subtilis*/*amyloliquefaciens* in its natural environment rely on the selective advantage conferred by the panoply of bioactive metabolites that it may produce. However, even if some strains are well equipped genetically to produce a vast array of antibiotics, only a limited part of this antibiotic-devoted genetic background may be readily expressed *in planta* and thus, only a part of this arsenal may be actually produced under natural conditions. The recent developments in biotechnology and analytical chemistry open the doors to new approaches for investigation and new tools to study *in situ* antibiotic production by valuable strains in their ecological niche. We will discuss how it could contribute to enhance our knowledge about *Bacillus* fitness in natural living conditions which is a crucial point for optimizing biocontrol strategies using this organism.

Submitted for oral presentation



A novel approach to protect *Bacillus thuringiensis* subsp. *israelensis* spores from the photo inactivation.

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Theme: Microbial metabolites

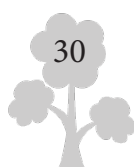
ORAL COMUNICATION

Abstract

Despite of all previous studies and report of change in climate and global warming in recent years, the solar inactivation of *Bti* have not deeply investigated, which is the major choice biopesticide for vector control in developing country. Especially, countries like India have average air temperature in month of April and May is above 40 °C. This may be the major problem for inactivation of biopesticide. To overcome such problems appropriate strategies are needed for development of potential formulations. A highly effective, long-term protective system against sunlight has not yet been reported for microbial insecticides. In this investigation some synthetic and microbial dyes were used as sunlight protectant for native mosquito larvicidal isolate *Bacillus thuringiensis* (BtSv2). The BtSv2 toxin without dyes after exposure of sunlight showed very high inactivation of toxin when tested against the deadly mosquito species, *Aedes aegypti* and *Anopheles stephensi*. While a microbial pigment, Prodiogiosin showed comparatively better result among the tested dyes in terms of toxicity. This may be due to tight binding of Prodiogiosin with the toxin or spores. It was also observed that the Prodiogiosin not only protect from sunlight but it also enhances the activity of *Bt sv2* by 6.16% against *Anopheles stephensi* and 22.16% against *Aedes aegypti*. This investigation suggests that protecting spores from inactivation by the visible wavelengths will probably extend the half-life of *Bt sv2* spores significantly, making it more effective as a microbial insecticide.

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Microbial lipopeptides : an emerging family of novel biopesticides

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In the framework of the European InterregIV Project PHYTOBIO (2010-2014), five universities and two applied research centers from France and Belgium have worked together to discover and develop new potential biopesticides. They focus their study on promising multifunctional biocompounds: microbial lipopeptides (1), all synthesized by the nonribosomal way. More than 30 researchers were involved in this common research and the following main results were obtained:

- Isolation and analysis of the mode of action of four new lipopeptides produced by *Pseudomonas* species: cichofactin and cichopeptin from *Pseudomonas cichorii* SF1-54 (2), sessilin and new orfamide derivatives from *Pseudomonas* CMR12a (3);
- Insights into the mechanism of perception by plant cell of surfactin, a lipopeptide produced by *Bacillus subtilis* able to induce systemic resistance. Surfactin perception relies on a lipid-driven process at the plasma membrane level (4);
- Potential of new antifungal mycosubtilin (another family from *Bacillus subtilis*) derivatives (5) to induce resistance mechanisms in grape wine cells (6);
- Demonstration of the plant protective effect of mycosubtilin and a mixture of surfactin and mycosubtilin on different pathosystems in greenhouse and in field trials (*Bremia lactucae*/Lettuce, *Fusarium*/leek, *Botrytis*/grape wine) (7, 8);
- Demonstration of the low ecotoxicity of mycosubtilin, surfactin or mixture of both (7);
- Analysis of the aggregation behavior of surfactin and mycosubtilin in aqueous solution and its consequence on their purification (9, 10);
- Production of the different lipopeptides in a new bioprocess involving membrane bioreactors (11).

In summary, this project has demonstrated for the first time the potential of lipopeptides as efficient biopesticides and the feasibility of their production in industrial bioprocesses.

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Endophyte screening from Indo-spanish medicinal plants: Biotechnological green crop protectants

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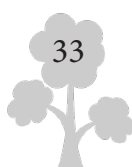
Endophytes are microorganisms that live in the intercellular spaces of plant tissues producing bioactive substances that may be involved in the host-endophyte relationship . These microorganisms represent a biotechnological source of bioactive metabolites.

As part of our ongoing study of the endophyte potential for the development of biotechnological biopesticides , we carried out the screening of endophytes from medicinal plants belonging to the genera *Laurus*, *Mentha*, *Artemisia* and *Thymus* present in India and Spain. Our study included the endophyte biodiversity (isolation and identification) of these plants species, the antagonism-based primary selection of isolates for microfermentation and the secondary selection based on their micro-extract activity on selected targets including insect pests, fungal pathogens and root-knot nematodes.

From a total of 11 plant species more than 1000 fungal strains were isolated and 700 were selected for microfermentation.

In this presentation we will discuss the relative percentage occurrence for each order and the colonization frequency for each fungal species. Additionally the antagonistic effects of these isolates against fungal pathogens (*Fusarium sp.*) and the bioactivity of their microextracts on the target species will be presented.

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Endosymbiotic actinobacteria from native plants of Algerian Sahara: biological control of the *Rhizoctonia solani* damping-off and tomato plants growth promoting activities

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Abstract

Thirty-four endosymbiotic actinobacteria were isolated from the roots of native plants of the Algerian Sahara. Morphological and chemical studies showed that twenty-nine isolates belonged to the *Streptomyces* genus and five were non-*Streptomyces*. All isolates were screened for their *in vitro* antifungal activity against *Rhizoctonia solani*. The six that had the greatest pathogen inhibitory capacities were subsequently tested for their *in vivo* biocontrol potential on *Rhizoctonia solani* damping-off in sterilized and non-sterilized soils, and for their plant-growth promoting activities on tomato seedlings. In both soils, coating tomato seeds with antagonistic isolates significantly reduced ($P < 0.05$) the severity of damping-off of tomato seedlings. Among the isolates tested, the strains CA-2 and AA-2 exhibited the same disease incidence reduction as thioperoxydicarbonic diamide, tetramethylthiram (TMTD) and no significant differences ($P < 0.05$) were observed. Furthermore, they resulted in a significant increase in the seedling fresh weight, the seedling length and the root length of the seed-treated seedlings compared to the control. The taxonomic position based on 16S rDNA sequence analysis and phylogenetic studies indicated that the strains CA-2 and AA-2 were related to *Streptomyces mutabilis* NBRC 12800T (100% of similarity) and *Streptomyces cyaneofuscatus* JCM 4364T (100% of similarity) respectively.

Keywords

Endosymbiotic actinobacteria, Biocontrol, *Rhizoctonia solani*, Plant growth promotion, Tomato

Biocontrol properties of Plant Growth-promoting Rhizobacteria

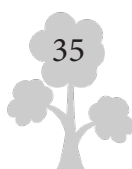
C. Prigent-Combaret

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The rhizosphere supports the development and activity of a huge and diversified microbial community, known as the rhizo-microbiote (Vacheron *et al.* 2013). Among the latter, plant growth-promoting rhizobacteria (PGPR) colonize roots of monocots and dicots and are able to promote the growth and health of plants by diversified mechanisms. Biocontrol PGPR can protect the plant through inhibition of phytoparasites including insects, based on antagonism or competition mechanisms, and/or by eliciting plant defenses such as induced systemic resistance (ISR; Couillerot *et al.*, 2009;). Biocontrol PGPR, especially from the *Pseudomonas* genus, display several relevant functional traits contributing to plant stress alleviation. Comparative genomic studies currently evidence the co-occurrence of biocontrol relevant properties in *Pseudomonas* PGPR genomes. Molecular exploration of rhizo-microbiotes to isolate new *Pseudomonas* strains harboring several distinct relevant biocontrol properties should allow the development of promising biocontrol inoculants.

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Suivi et impact de *Rhodococcus erythropolis* R138, agent de lutte biologique ciblant les bactéries responsables de la jambe noire sur culture de pomme de terre

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Chaque année en France, les bactéries des genres *Pectobacterium* et *Dickeya* responsables de la maladie de la jambe noire causent une perte d'environ dix mille tonnes de plants de pomme de terre (Assemblée générale FN3PT, 2013). La virulence de ces bactéries est régulée par Quorum Sensing (QS), système de communication cellulaire axé sur la reconnaissance et la concentration de molécules signal de la famille des Acyl Homoserine Lactone (AHL). Actuellement, il n'existe aucun traitement contre cette maladie. Une stratégie de lutte développée au laboratoire (Quorum Quenching) consiste à introduire la souche *Rhodococcus erythropolis* R138 en tant qu'agent de bioprotection (Uroz et al., 2003). Cette bactérie isolée du sol est capable de dégrader les signaux Quorum Sensing et ainsi perturber la virulence des bactéries pathogènes. Afin de suivre les populations de R138 et du pathogène *Pectobacterium atrosepticum* CFBP 6276, leurs génomes ont été séquencés et des marqueurs moléculaires souche spécifique ont été développés. Un test de protection en champs a ensuite été réalisé à la station expérimentale du Comité Nord/SIPRE à Achicourt (62). Cet essai a permis dans un premier temps de suivre l'évolution des populations de R138 et de Pa6276 dans la rhizosphère et dans les plants de pomme de terre. Dans un second temps, l'efficacité de cette stratégie a été évaluée en réalisant une cinétique d'apparition de symptômes de jambe noire. L'expérimentation en plein champ permet de montrer que l'introduction de R138 couplé à un biostimulant permet son installation durable dans le sol rhizosphérique mais ne permet pas toujours une protection significative vis-à-vis de l'apparition des symptômes sur plantes. En complément de ces travaux ciblés sur la virulence de *Pectobacterium*, une seconde stratégie de biocontrôle est développée au laboratoire et met en jeu d'autres agents de bioprotection agissant par antibiose sur les deux genres pathogènes *Pectobacterium* et *Dickeya*. Cette nouvelle approche fait l'objet d'essais en serre (pathosystème) et au champ.

Non-ribosomal Peptides from Entomogenous Fungi

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Abstract: Entomogenous fungi play a very important role in controlling natural population of insect pests. Many of them have been developed as myco-insecticides. In long time evolution, entomogenous fungi acquired lots of pathogenic factors against host insects. Non-ribosomal peptide (NRP) is a usual pathogenic factor of entomogenous fungi. Chemically, NRPs are the secondary metabolic compounds mainly composed of specific or modified amino acids and hydroxyl acids. They are synthesized via thio-template multienzyme mechanism of multifunctional enzyme complex system other than on ribosome. NRPs show a broad range of biological activities such as insecticide, antibiotics, antiviral, antitumor, etc. Meanwhile, researchers have been focusing the interests on the NRPs properties as immunity inhibitor, cell proliferation inhibitor, siderophore and ionophore, etc. To date, more than twenty kinds of NRPs were isolated and identified from entomogenous fungi genera: *Beauveria*, *Conioideocrella*, *Cordyceps*, *Culicinomyces*, *Fusarium*, *Hirsutella*, *Isaria*, *Metarhizium*, *Paecilomyces* and *Verticillium*, etc. These NRPs include bassianolides, beauvericins, beauverolides, beauveriolides, cicadapeptins, conioideocrellides, cordycommunins, cordyheptaepptides, culicinins, cyclosporins, destruxins, efrapeptins, enniatins, hirsutellides, hirsutides, isariins, isaridins, isarolides, paecilodepsipeptides, serinocyclins, etc. The structures, activity and mechanism of these NRPs were reviewed in this chapter.

Key words: entomogenous fungi; non-ribosomal peptides; structure, activity, mechanism.



Biocontrôle de la pourriture brune du cacaoyer par des *Trichoderma* Etude des métabolites et de leur détoxification

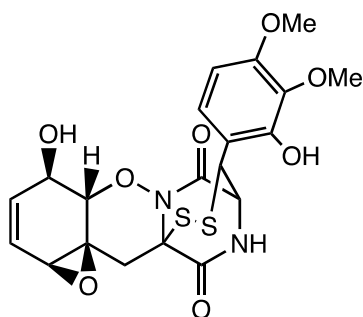
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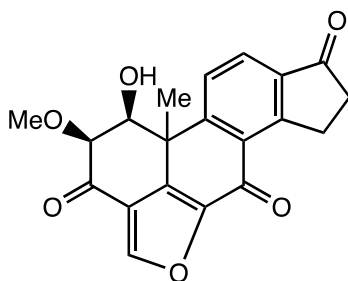
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Le cacao est produit à plus de 70% par des pays d'Afrique occidentale et centrale. Malheureusement cette production est affectée par la pourriture brune des cabosses due à des espèces de *Phytophthora*¹ qui occasionnent chaque année, des pertes estimées à 44 % de la production mondiale. Bien qu'efficace, la lutte chimique reste contraignante, onéreuse et présente des risques énormes d'intoxication pour les utilisateurs. Le biocontrôle par l'utilisation de microorganismes antagonistes est devenu une alternative prometteuse de la stratégie de lutte intégrée contre cette maladie en phase d'expansion. Les recherches effectuées au Centre National de Recherche Agronomique de Côte d'Ivoire ont permis l'isolement et la sélection de 3 isolats de *Trichoderma* ayant montré un pouvoir antagoniste élevé contre *P. palmivora*².

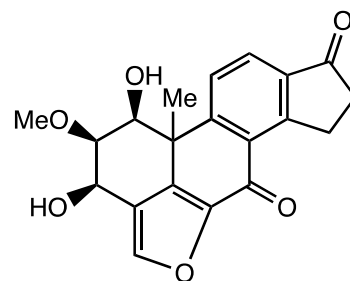
Nos travaux sont la continuité de cette étude et nous nous sommes intéressés à l'aspect moléculaire de cet antagonisme. Parmi les 14 métabolites isolés de cultures de ces champignons, deux métabolites, la gliovirine 1 et la viridine 2, ont montré de fortes activités antifongiques. Nous avons évalué les activités de ces molécules sur la germination des zoospores et contre la croissance mycélienne de plusieurs espèces de *Phytophthora* responsables de la pourriture brune du cacaoyer. Ces évaluations biologiques nous ont permis de mettre en évidence l'action synergique de ces deux molécules. Enfin nous avons montré que plusieurs microorganismes de l'environnement sont capables de réduire la viridine en viridiol 3, composé sans activité antifongique. Cette détoxification de la viridine montre les limites du biocontrôle dans la mesure où le viridiol est une phytotoxine.



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Marine-derived *Trichoderma* strains producing peptaibols as a potential source of new biocontrol agents.

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Currently, biocontrol agents represent only a small part of the world market of plant protection and rely on a reduce number of biological agents and substances. In this context, we propose to search new biocontrol agents from the marine environment, particularly fungi. Studies in the field of biocontrol on marine fungi are almost inexistent, but everything suggests that the marine ecosystem could represent a significant source of new biocontrol agents (Gal-Hemed et al., 2011). In fact, this environment contains an incredible microbial diversity, synthesizing molecules with different biological properties. Moreover, even though many fungi have a terrestrial origin, they are biologically and biochemically acclimated to the conditions of marine life and can produce an original metabolome. In addition, marine fungal strains are generally very resistant to various environmental stresses and therefore have a great potential for agricultural use.

The marine fungal strain collection of the MMS laboratory includes more than 900 isolates from shellfish and sediments of the French Atlantic coast. In this collection, several genera are present. Genus *Trichoderma* is one of the most important in number of strains. Different *Trichoderma* strains from terrestrial origin are already used in biocontrol. Their marine-derived homologs could then constitute a potential source of new bioactive agents. To select marine strains with high potentiality for biocontrol, we are developing an original methodology to select the most effective agents.

The majority of the *Trichoderma* spp. strains are producing, peptaibols, small antimicrobial peptides, which have been described to play a role in the elicitation of induced systemic resistance in plants (Viterbo *et al.*, 2007; Degenkolb *et al.*, 2006). A study on the activity of the different peptaibols and the relationship between their structure (chain length, polarity...) and their antimicrobial activity can be one of the objective criteria for the new approach to screen strains of *Trichoderma* on the basis of their peptaibols production profile. An original laser nephelometry technique was used to study the filamentous fungal growth and was applied to estimate the antimicrobial properties (Joubert *et al.*, 2010).

The present work describes the first results on peptaibol activity and their chemical quantification as criteria for selection of marine-derived *Trichoderma* strains as putative new biocontrol agents.

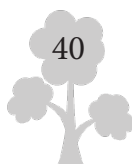
Procédés de Fermentation en milieu solide et bioreacteur à usage unique pour la production de biopesticides

S. Roussos , M.S. Tranier, J. Pognant-Gros, Q. Carboué, M. Mahjoub, I. Perraud-Gaime

Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), AMU Campus étoile, FST St Jérôme 13397 Marseille cedex 20 - France. La Fermentation en Milieu Solide (FMS) est un procédé permettant le développement du micro-organisme à la surface et à l'intérieur d'une matrice poreuse solide et humidifiée, en absence d'eau libre. De ce fait, les champignons filamenteux conviennent particulièrement bien pour ce type de culture, grâce à leur capacité de colonisation des surfaces et leur faible besoin en eau ($0.6 < A_w < 0.9$). Les procédés de FMS sont agités ou statiques, se réalisent sur des substrats solides ou sur des supports naturels ou synthétiques et permettent la production de biomasse, d'enzymes, de métabolites primaires et secondaires. Les bio réacteurs utilisés sont des dispositifs du laboratoire ou des dispositifs industriels permettant de réaliser des cultures des champignons filamenteux dans des conditions expérimentales bien définies et souvent les principaux paramètres de culture (température, humidité, pH, CO₂) sont pilotés. Plus récemment des nouveaux bio réacteurs en milieu solide à usage unique (FMS-unique) ont été mis au point et brevetés par l'IRD (Roussos et al., 2007 ; 2013). On présentera un dispositif de 40 litres, pouvant contenir 4 kilos de matière sèche, qui a été dessiné pour effectuer la production de biopesticides à l'échelle pilote industriel. Le FMS-unique est constitué de deux parties : une chambre basse en PVC qui sert pour l'aération des cultures et une chambre haute appelée « chambre de fermentation » qui reçoit les substrats solides humidifiés et inoculés pour la culture des champignons filamenteux. Dans le procédé IRD, la culture des microorganismes se réalise sur un mélange de sous produits agroindustriels (bagasse de canne à sucre, son de blé, purée de pomme de terre, grignons d'olive, déchets de crevette) humidifiés à 75% avec une suspension de conidiospores. Les milieux de culture sont introduits dans des bioréacteurs et l'aération forcée avec de l'air humide s'applique pendant 72 heures à 27°C. Après 72 heures d'incubation, l'aération se réalise avec de l'air sec à 25°C, permettant d'exercer un stress hydrique afin d'une part maintenir la phase de sporulation pendant plusieurs jours et d'autre part, obtenir un produit final sec à moins de 5% d'humidité et contenant plus de 4×10^9 spores par gramme de poids sec du produit fermenté et virulent. Le bioréacteur FMS-unique de 40 litres, a une capacité de production de 1013 spores par cycle de production. Cette quantité de spores est suffisante pour lancer des fermentations avec 1 à 10 tonnes de substrats poids sec. Également il permet de fournir des biopesticides (*Trichoderma*, *Paecilomyces*, *Metarhizium* *Microdochium*) pour traiter environs 50 hectares de culture sous serre. Il s'agit d'un nouveau concept de bioréacteurs à usage unique, permettant d'une part, de réaliser toutes les opérations unitaires de traitement de substrats, d'incubation et d'autre part, d'exercer un stress hydrique pour favoriser la sporulation et à la fin du procédé d'obtenir un produit sporulé sec pouvant être conservé, à la température ambiante pendant plusieurs mois dans le même emballage, sans perte de viabilité et de virulence,

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Olfactory signal extraction from noisy environment: how moths respond to pheromones in a plant odour background

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Olfaction is an essential sensory modality to insects that use semiochemicals to find a proper mate, a food source or a suitable oviposition site. These air-borne chemical signals have a high potential in plant protection because of their non toxic mode of action, their specificity and their proven efficiency for manipulating behaviour. In turn, compared to biocides, semiochemical based strategies necessitate a good understanding of insect sensory ecology and being aware of plasticity in insect responses. Insect olfaction has received significant research effort. Considerable progresses in analytical chemistry have resulted in the identification of a great number of organic volatile compounds in spite of the difficulties caused by their generally very low amounts and complexity of mixtures. In parallel to signal characterization, our knowledge of the molecular and neuronal processes underlying insect olfactory-driven behaviours has also significantly progressed.

Although the capacity of learning new odours has been studied in bees and moths, it is more often considered from many case studies that insects respond in a rather deterministic way to specific signals released by host-plants or con-specific. However in natural conditions, even specific chemical signals must be discriminated from a complex and fluctuant environment so that the information content of the signal might be degraded. First, we have learned that air-borne signals once dispersed by turbulent air flows appear as highly intermittent filaments. Flying insects thus experience alternations of non-odorized and odorized spaces with low reliability regarding the direction of the source. In turn, although global concentration of odorants rapidly decreases, relatively high concentration of odorant may be present within “filaments” at a distance from the source. The physical structure of the signal is important for the insect orientation. Second, the insect must extract the relevant information, generally a blend of several components in precise ratio, from a huge diversity of other volatile organic compounds. Olfaction is generally known as a synthetic modality (i.e. animals perceives blend as a whole) but animals must extract this whole from an “olfactory cocktail party” comprised of many more volatile organic compounds, some of them carrying ecologically relevant information. The patterns of signal and background odorants arrival on the insect receptors are also affected by the physical structure of air-borne signals.



The system plant odour/pheromone is a suitable model to address the question of the perception of signal against background. Male moths recognize and localize conspecifics female by a sex pheromone released by female. Both sexes also respond to flower volatiles, as indicator for adult food sources, or to larval host-plant emissions. It is generally admitted that pheromones are detected and processed in a separate sub-system from general odorants in the male olfactory system. Specialized olfactory receptors expressed in specialist olfactory neurones housed in long hairs on the antennae act as molecular filter towards the components of pheromone blend. Each type of pheromone olfactory neurones projects in a sexually dimorphic area of the male brain. General odorants are processed in separate areas of the antennal lobes. Pheromone blend triggers in the male an oriented flight. However, addition of plant odorant has been shown to increase male attraction to pheromone. Using complementary approaches we have shown that general odorants may interfere with pheromone perception. Such interactions affect detection by receptor neurons resulting in alteration in intensity, temporality and ratio coding of the pheromone signals. Partial agonism, antagonism or sensory masking may be observed in the responses of receptor or central neurons. Male moths orienting to a pheromone source temporarily modify their locomotion pattern in response to a change in olfactory background.

Our findings indicate that perception of a chemical signal is largely dependent on the context, even in a very determined communication system. Interactions between signal and background odours appear as important factors of variability within natural communication webs. On a practical point of view, applications of pheromones make largely use of synthetic lures for mass trapping or monitoring purpose. Interactions between released semiochemicals and background odorants can affect positively (synergy) or negatively (inhibition) the efficiency of these methods. In addition, a better understanding of the process of signal extraction in noisy environments appears even more relevant when protection involves deterring insects from crop plants or livestock by chemical masking of attractive odours.

Sustainable plant protection strategy for artichoke downy mildew

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To face downy mildew (*Bremia lactucae*), major disease on artichoke, Vegenov is working with its field partners on the development of environmentally friendly solutions. We evaluated “low risk” products under controlled conditions and identified eight of them showing good protection efficiency (between 50 and 80%). Field assays will be conducted in order to confirm their interest for producers. In parallel, a prediction model for disease development has been developed in order to adapt the positioning of treatments to disease risk. It gave satisfying results, as the model could predict the appearance of the disease for 85% of the field parcels evaluated. Following this work, we plan on combining efficient “low risk” products and naturally resistant varieties in order to study the potential cumulative effect of those strategies.

Elicitor screening to protect winter wheat against *Septoria tritici* Blotch: preliminary results

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Elicitors are plant immunity-triggering compounds which are currently considered as one of the most promising tools in agriculture for the induction of plant resistance to various diseases (Mejía-Teniente *et al.*, 2010). By contributing to both economic and environmental performances of agroecosystems, they can help reducing the use of chemical inputs. Although some elicitor products are already available on the market, it appears that a variable efficiency in the field, along with an uneasy integration in the current legislation and agricultural strategies, make these tools difficult to use (Walters, Ratsep, & Havis, 2013). Besides these limitations, few elicitor treatments have yet been efficiently and specifically designed to protect crop plants such as wheat, which is grown and consumed worldwide, against major diseases threatening both their yield and quality. There is a strong need to better understand the mechanisms of induced resistance in plants and develop elicitor use in agriculture.

A PhD research is currently led in Gembloux Agro Bio-Tech to develop a method based on eliciting agents, tested in greenhouse and field conditions, to protect winter wheat against major diseases, namely *Septoria tritici*, *Fusarium graminearum* and *Fusarium culmorum*. This project focuses on the screening of a large number of elicitors from different origins and structures.

In 2014, we focused the first screening experiments on the protection of wheat against *Septoria tritici* Blotch (STB). Two winter wheat genotypes were tested: susceptible 'Avatar' and semi-resistant 'Sy Epson'. Plants at 3-leaf stage were first sprayed till runoff with different concentrations of elicitors, and then inoculated 5 days later with a *Septoria tritici* spore suspension (10^6 spores mL⁻¹) using a hand sprayer. Control plants were treated, prior to disease inoculation, with sterile water (negative control) or with BION® (ASM, Syngenta Europe; positive control). The disease severity and incidence were scored every 2 days for 28 days post-inoculation (d.p.i) by measuring the percentage of area covered with lesions and bearing pycnidia on the third leaf.

The first results of these tests should enable a first discrimination of elicitors considering their dose-efficiency to reduce disease severity. After further screening of all the elicitors in hand, a determination of the elicitation pathways of the most efficient molecules tested will be undertaken. We intend to study the influence of various factors (i.e Temperature, relative humidity, plant development stage) on the elicitation potential and develop a formulation to be finally tested under field conditions.

Keywords: Winter Wheat, Elicitors, Screening, *Septoria tritici* Blotch

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Evaluation de l'efficacité des différents biofertilisants sur les populations résiduelles du puceron noir de la fève *Aphis fabae*.

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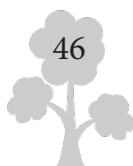
Résumé

La sévérité causée par les pucerons (Homoptera: Aphididae), rend surtout nécessaire la mise au point de solutions alternatives s'appuyant sur le rôle des biofertilisants comme éliciteurs naturels dans la stimulation des défenses naturelles des plantes avec l'introduction de nouvelles formulations pour faire face aux bioagresseurs. L'étude a porté sur la stimulation des défenses naturelles de la fève *Vicia fabae* L par l'utilisation des différents biofertilisants (jus de lombricompost (brut et formulé)), jus de lombricompost fermenté (brut et formulé) et l'extraits des algues marines. L'effet des différents biofertilisants a été évalué sur des populations résiduelles de puceron noir *Aphis fabae*.

Les résultats de l'évolution temporelle des populations résiduelles d'*Aphis fabae* montre que les produits appliqués présentent une efficacité importante pour le jus de lombricompost brut et formulé et le jus de lombricompost fermenté brut et formulé et modérée pour l'extraits des algues marines, et une rémanence assez longue pour le jus de lombricompost brut et formulé et le jus de lombricompost fermenté brut et formulé et une reprise biocénotique des populations résiduelles du puceron de la fève accès faible. Sur le plan effet jumelé, le jus de lombricompost formulé affiche un meilleur effet en développant un effet SDN. Ces mécanismes de défense des plantes inductibles par des bioagresseurs impliquent par exemple des molécules à action antimicrobienne ou insecticide directe ou encore des molécules impliquées dans le renforcement des parois cellulaires, freinant ainsi la colonisation. La connaissance approfondie de ces mécanismes de défense a permis le développement et l'utilisation des biofertilisant qui n'agissent plus directement sur la cause du stress, mais qui présentent la propriété d'agir indirectement par la stimulation des mécanismes de défense naturelle. Ces produits appelés « Stimulateurs de Défense Naturelle - SDN ».

Mots clés

Population résiduelle, algue marine, *Aphis fabae*, biofertilisants, jus de lombricompost, jus de lombricompost fermenté. *vicia fabae*



Activity and characterization of hypoiodide and hypothiocyanate ions, two active molecules against plant pathogens

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SUMMARY

The lactoperoxidase system (LPS) is a natural biological protection system present in mammalian's glands such as salivary glands, lacrimal glands, airway mucus, vaginal secretions, intestinal secretions and milk. LPS produces hypothiocyanite (OSCN-) and hypoiodite (OI-) or iode (I₂) depending of the pH. These molecules are described as having antibacterial, antifungal and antiviral properties [1]. In this context, OSCN- reacts specifically through oxidation of -SH moiety. OSCN- is measured with a colorimetric method based on the oxidation of SH group. The same method can be used for measuring OI- which is able to oxidize thiol, aromatic cycles, thioethers and amines. In solutions containing both molecules, this method is not able to measure specifically each molecule although the characterization of each active agent is necessary for agricultural applications and homologation procedures.

We have developed three aqueous solutions containing i) only the active agent OSCN- or ii) OI- or iii) both ions OSCN- and OI-. The solutions were tested through in vitro ELISA tests against potatoes, wheat, tomatoes, orchards plant pathogens and against postharvest pathogens of citrus fruits, apples, pears, bananas and potato tubers. The three solutions have different characteristics regarding their color, their concentration and *in vitro* efficacy. Solution containing OSCN- and OI- inhibited, with inhibition range from 70% to 100%, 16 pathogens on 17 tested although solely OSCN- or OI- ions solutions inhibited only 3 pathogens on the 9 tested.

The characterization of the active agent in each solution was realized through the development of an easy specific dosage method of OI- or I₂ [2] and ultrafiltration.

Furthermore *in-vivo* trials shows high efficacy of OSCN- and OI- solutions in post-harvest applications such as in apples against *Penicillium expansum* and *Botrytis cinerea*; in citrus fruits against *P. italicum* and *P. digitatum* and in potato tubers against *Pectobacterium carotovorum*. In cultivated plants, OSCN- and OI- solutions shows high inhibitory activity against *Plasmopora viticola* in grapevine crops and against *Phytophthora infestans* in potatoes crops.

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Utilisation de micro-doses de sucres en protection des cultures.

Synthèse du projet Usage.

I. Arnault, S. Ondet.

L'objectif du projet est de mettre en application une méthode innovante induisant la résistance des plantes aux bio-agresseurs en agriculture biologique sur 3 filières végétales. La méthode consiste en l'application foliaire d'infra-doses de composés utilisés par la plante et jouant un rôle dans la reconnaissance entre la plante et le phyto-agresseur. Ces composés, des diholosides et hexoses (glucose, fructose, saccharose, tréhalose) sont des métabolites primaires de la plante. La méthode induit des changements métaboliques dans les tissus et à la surface des feuilles de plusieurs espèces végétales, ainsi qu'un effet rapide sur l'expression de gènes impliqués dans les systèmes de protection contre les stress. Les effets d'induction de résistance de la plante sont similaires à ceux des éliciteurs de défense, cependant d'autres voies de signalisation non communes aux éliciteurs semblent être activées avec un effet plus immédiat. Des résultats de laboratoire suivis d'application du saccharose contre le carpocapse en verger de pommier montrent depuis 4 ans des réductions notables des dégâts avec méthode. Ce projet souhaite transférer la méthode dans les itinéraires culturels des arboriculteurs et investiguer son efficacité en viticulture et en maraîchage en situations agronomiques très critiques vis-à-vis de certains bio-agresseurs.

Après 3 ans d'essais, le projet a permis d'apporter de nouveaux résultats.

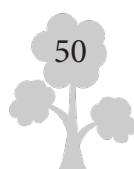
- Le fructose 100 ppm, saccharose seul ou association avec des produits présente des résultats significatifs contre le mildiou de la vigne, la pyrale du melon et le carpocapse des pommes et des poires. Le glucose et tréhaloses testés en conditions contrôlées et semi contrôlées n'ont montré aucune efficacité. Le fructose pourrait permettre de réduire les doses de cuivre mais l'effet semble conditionner aux variétés et cépages étudiés.

- Pour les autres cultures et pathogènes, comme la salade/*Botrytis*, pomme de terre/mildiou, nous n'avons pas obtenu de résultats convaincants. Les pathogènes ciblés d'importance économique sont trop difficiles à contourner avec une action des sucres supposée « priming ». Par ailleurs, des champignons comme *Botrytis* pourraient être favorisés par les sucres, comme le glucose.

- De façon très nette, ce projet a permis aussi de mettre en évidence un effet variétal sur l'efficacité de l'application des sucres sur pommier et vigne. Par ailleurs, ces sucres semblent présenter des résultats intéressants en cas de pression modérée des bio-agresseurs. Lorsque la pression est forte, la protection semble décrocher.

Le fructose donnant des résultats intéressants, le dossier d'homologation du fructose a été entamé en 2013, pour la partie scientifique et pour les impacts toxicologiques et environnementaux.

Posters



Chemical characterization of the nematocidal agents from *Artemisia absinthium* hydrolate

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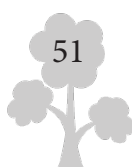
Artemisia absinthium L. (wormwood) is an aromatic and medicinal plant of ethnopharmacological interest. The composition and biological effects (antimicrobial antiprotozoal, acaricidal, insecticidal, antifungal) of potentially toxic thujone-rich essential oils (EO) of *A. absinthium* have been widely studied.

Spanish populations of wormwood have been domesticated for experimental cultivation (Burillo, 2009; Bailén et al 2013; Gonzalez-Coloma et al., 2012; Martín et al., 2011). The biological effects (insect antifeedant action and antioxidant effect) and constituents of the EOs, ethanolic extracts (OSEs) and supercritical fluid extracts (SEs) of these two populations have been described, with the sesquiterpene lactone hydroxypelenolide being the major component of OSE followed by the flavones artemetin, and casticin. The EOs were characterized by the presence of cis-epoxyocimene, chrysanthenol, linalool and chrysanthenyl acetate among others. Samples rich in cis-epoxyocimene and sesquiterpenes were the most active ones against insects and fungal pathogens. SE extracts showed an improvement in the yield of several mono- and sesquiterpenes and were more active than the traditional ones (EO, OSE) against insects. Therefore, thujone free *A. absinthium* plants (registered plant variety EU 36714, 2014) are being developed for the production of botanical biopesticides (PCT/ES2012/070162. Ref. 201031389; PCT/ES2012/070162. Ref. 300055694).

The pilot-plant steam distillation of cultivated *A. absinthium* generates EO and hydrolate (WR) as byproduct. This WR was nematocidal against *Meloidogyne javanica*. The organic extract (OE) of this WR obtained by solid phase extraction showed strong nematocidal activity. The bio-guided study of the chemical composition of the OE by flash chromatography and HPLC resulted in the isolation of a number of monoterpene compounds, including two diols also present in the EO, along with several new monoterpene alcohols. These compounds were identified by spectroscopic techniques (1D and 2D NMR) and mass spectrometry (GC-MS and HPLC-MS).

In this presentation we will discuss the potential development of *A. absinthium* WR byproducts as nematocidal agents.

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Biocidal effects of extracts from *Artemisia assoana* cultivated under different environmental conditions

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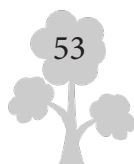
⁴ Instituto de Productos Naturales y Agrobiología, IPNA-CSIC. Avda. Astrofísico F. Sánchez, 3, 38206. Tenerife, Spain.

The genus *Artemisia* L. (Asteraceae) comprises about 500 species mostly distributed in the Northern Hemisphere (Bora and Sharma, 2011). Some of the species of this genus have shown to be antiparasitic, antihelmintic, acaricidal and insect repellent (see Gonzalez-Coloma, 2012).

As part of our ongoing taxonomically-oriented bioprospection of *Artemisia* sp (Gonzalez-Coloma, 2012) we have selected *A.assoana*, a rare plant with an Ibero-Mediterranean distribution that grows in degraded grazed land in continental climate at 900–2000 m. *A. assoana* has been reported to contain phenolic and acetylenic metabolites (Martínez et al., 1987). Similarly, artificially cultivated *A.granatensis* has been reported as a strong aphid-repellent based on its content in poliacetylenic spiroacetals (Barrero et al., 2013). Plant material and cuttings were obtained from a wild population growing in Teruel (Spain). These cuttings have been kept in a greenhouse and further multiplied to establish artificial (Aeroponic and in vitro transformed root), greenhouse and field cultivations. Volatile (Clevenger distillation) and organic extracts (Sohxlet ethanolic extraction) have been obtained from wild aerial, aeroponic aerial and root, in vitro transformed root, and field aerial parts and the extracts have been tested against herbivorous insects (*Myzus persicae*, *Rhopalosiphum padi* and *Spodoptera littoralis*), plant parasitic nematodes (*Meloidogyne javanica*), plants (*Latuca sativa* and *Lolium perenne*) and the insect vector *Rhodnius prolixus*, (vector of Chagas disease). Additionally, the trypanocidal activity of these extracts on epimastigote forms of *Trypanosoma cruzi* Y strain has been tested. In this presentation we will discuss on the sustainable production of *A. assoana* plant biomass to generate bioactive extracts. The metabolic profiles of these extracts will be correlated with their bioactivity.

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A simple saving-compound-method to study insecticidal activity of natural substance extracts and associated molecules on mosquito larvae.

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The research on new natural insecticides has intensified with the spread of insect resistance to synthetic chemicals¹. Screening extracts and molecules against adult and immature stages of disease vectors is largely done by following standardized protocols as described in World Health Organization manuals². Other researchers have developed their own high-throughput-system to increase their screening capacity because finding efficient candidates lead to test a large span of compounds, often in relatively important amount³. Therefore, the challenge is to find a sensitive method and assay system, efficient for a large range of extracts and molecules and which is able to save testing time and compounds.

Our project aims at searching and characterizing new natural insecticides in the diversity of Amazonian plants of French Guiana, by assessing the larvicide activity of extracts originating from more than 80 plant species on a susceptible *Aedes aegypti* laboratory strain. Then, each active extract (more than 50 % mortality at 100 ppm on the laboratory strain) was tested on a pyrethroid resistant strain. Bioguided fractionation of the most active extracts was then performed, and the larvicidal activity of isolated compounds was evaluated on both strains. However, this methodology is compound-consuming, and extracts and pure compounds are often produced in limited quantity. Therefore, we undertook to develop an assay that permitted to reduce the quantity of compounds involved without losing the sensitivity of the WHO larval method.

After testing various containers of different sizes and matter, 5 ml glass tubes were selected. We present here an optimized assay by comparing this assay to the WHO larval test for synthetic (dichlorvos, malathion propoxur, deltamethrine) and natural (spinosad, pyrethrum) chemicals, extracts (*Muelleria frutescens*, *Sextonia rubra*) and their associated active molecules (rotenone, rubrenolide, rubrynlolide). Pearson correlation test of LD₅₀ and LD₉₀ at 24 and 48h revealed a strong correlation (R^2 in the 0.68-0.96 range, $p < 0.001$) between the data from cups and tubes. We also determined that 10 tubes (N=50 larvae) provide the same level of sensitivity than 20 tubes (N=100). This new method is thus suitable for rapid screening of a large number of natural extracts and molecules to identify effective compounds, and to precisely measure LD₅₀ or LD₉₀, using 8 times less compound than the WHO protocol.

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Antifeedant activity from benzofurane-type compounds

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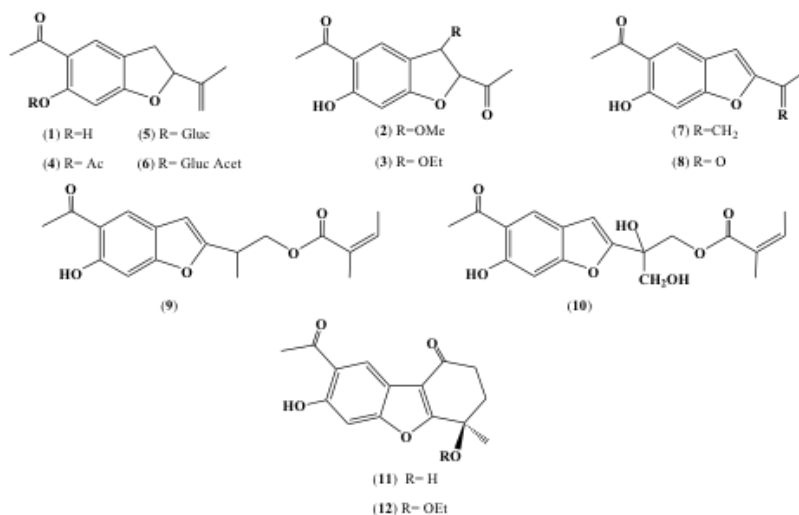
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Benzofuranes are characteristic natural compounds in many plant families as *Rutaceae*, *Liliaceae* or *Ciperaceae*, but mostly have been isolated from certain tribes of *Asteraceae* family.¹⁻², Several benzofuranes have shown interesting biological activities³⁻⁴.

As part of our research in biopesticide models, we report the isolation of bioactive benzofurane-type compounds from two species of *Pericallis*, the only genus in the tribe *Senecionae* endemic of the Macaronesian region. We collected aerial parts and seeds of *Pericallis stettzii* and *P. echinata* wild populations and established in vitro transformed root cultures (*Agrobacterium rhizogenes* ATCC 15834) for the sustainable production of plant biomass. We obtained derivatives 5 and 6 from the microbiological transformation of the mayor component 1 with *Mucor plumbeus*.

Products 1-12 were identified by RMN as benzofurane-type compounds. Two of them are new natural products (9 and 10). All were bioassayed as insect antifeedants against several pests with different feeding ecologies (*Leptinotarsa decemlineata*, *Spodoptera littoralis*, *Myzus persicae* and *Rhopalosiphum padi*).



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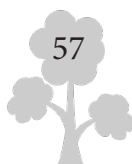
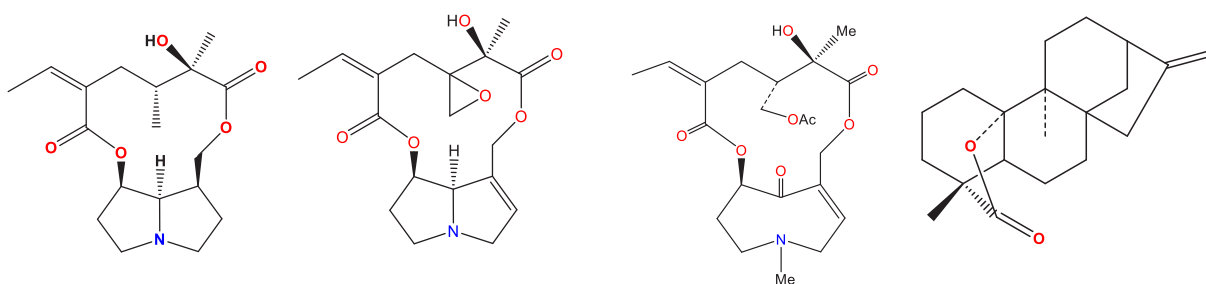
Bioactive compounds of the genus *Senecio* from Peruvian Andes

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The genus *Senecio*, is widely distributed throughout the world and consists of approximately 1500 species¹. This genus is characterized by the presence of pyrrolizidine alkaloids (PAs)², sesquiterpenes and diterpenoids³. Furthermore, PAs are sequestered by specialist insects for their defense and chemical communication and are also considered plant defenses against generalist herbivore insects⁴. About 230 species of this genus are present in the Peruvian flora, and several are frequently used in traditional medicine for the treatment of diverse diseases⁵.

In the present work we report on the isolation, structure determination of PAs,^{6,7} ^{8,9,10,11} and diterpenoids^{12,13,14,15} isolated from native Peruvian *Senecio* species. Platyphylline (1) and platyphylline N-oxide (2) were isolated from *S. lasiocephalus*; 13,19-epoxysenecionine (3) from *S. usgorensis*, senecivernine (4) from *S. chiquienensis*, 19-acetoxisenkirine (5) and senecionine (6) from *S. lariciphilius*; senecionine (6), senecionine N-oxide (7), retrorsine (8) and seneciphylline (9) from *S. subcandidus* leaves and stems parts. The diterpenes ent-kaur-16-en-19-oic acid (10), (-)-Kaur-16-en-19-ol (11), (-)-kauranol (12) and tetrachyrin (13) from *S. klugii*. Their structures were established using mono-bidimensional NMR spectroscopic techniques. The defensive properties of these extracts and compounds have been tested against several herbivorous insects [*Spodoptera littoralis*, *Mysus persicae* and *Rhopalosiphum padi*],^{10,14} and their phytotoxic effects against *Lolium perenne* and *Lactuca sativa*.



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Cultivated *Geranium macrorrhizum* L. as a source of germacrone-based bioactive extracts and compounds

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Geranium macrorrhizum is a perennial plant species in the Geraniaceae family. It is native to the South east Alps and the Balkans and cultivated as an ornamental plant. The plant is used for medical purposes in the traditional medicine due to its antimicrobial properties [1]. A major component of the essential oil is the sesquiterpene germacrone [1,2]. Additionally, it contains flavonoids and phenolic acids and the insecticidal activity of the leaf extracts has been reported [3,4].

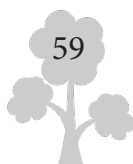
Given the germacrone content of *G. macrorrhizum* and its potential applications, we have started its experimental cultivation in Spain (CITA-Aragon). Cuttings from two cultivated populations (Hungarian and English) were planted in pots and kept under different environmental conditions (green house, open). Germacrone-rich extracts (Essential oil, EO and hexane, Hx) have been prepared from the two populations to compare their chemical profiles and their effects against insect pests (*Spodoptera littoralis*, *Myzus persicae*, *Rhopalosiphum padi*) and ticks (*Hyalomma lusitanicum*). In this presentation we will compare the chemical composition and biological effects of extracts (EO, Hx) and fractions of *G. macrorrhizum* along with germacrone and derivatives.

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Efficacité comparée d'huiles essentielles formulées à base de Thym et d'Origan sur l'abondance de deux pucerons d'Agrumes

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Résumé

Actuellement, les plantes aromatiques possèdent un atout considérable grâce à la découverte progressive des applications de leurs huiles essentielles dans la lutte biologique contre les ravageurs des cultures. La présente étude a porté sur l'évaluation de l'efficacité des huiles essentielles formulées à base de Thym et d'Origan comparée à un produit de synthèse (Methomyl) sur l'abondance de deux pucerons d'agrumes (*Aphis citricola* et *Aphis gossypii*). Les résultats de cette étude ont montré que toutes les molécules testées ont eu un effet répressif sur les ravageurs ciblés avec une suprématie d'efficacité de l'huile essentielle à base de thymol par rapport aux autres biopesticides appliquées. En revanche le thymol reste moins efficace que le produit chimique qui affiche le plus faible taux d'abondance des populations aphidiennes. Ces mêmes résultats ont permis, de mettre en évidence un effet choc et une toxicité temporelle de toutes les molécules testées. L'estimation de l'efficacité des doses appliquées, a dévoilé que toutes les doses ont eu une répression sur l'abondance des populations des pucerons visés mais les doses complètes (D) se révèlent nettement plus efficaces que les demi-doses (DD).

Mots clés : Abondance, Biopesticides, Carvacrol, Huiles essentielles, Methomyl, Origan, Pucerons, Thymol, Toxicité.

Etude de la toxicité d'huile essentielle d'origan sur le *Varoa jacobsoni* parasite d'*Apis mellifera* dans la région de la Mitidja

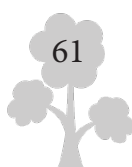
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Laboratoire de plantes aromatiques et médicinales. Département des Biotechnologies. Université Sad DAHLEB Blida. Algérie.

Résumé :

L'effet toxique d'huiles essentielles de plusieurs plantes sur le genre *Varoa* a été déjà décrit par plusieurs auteurs. Cependant, peu d'entre elles ont été vraiment efficace. Dans la présente étude, nous avons opté pour la mise en évidence de l'effet d'huile essentielle d'*Origanum vulgare*, suite aux plusieurs essais entrepris aux champs par nos apiculteurs sur des ruches d'abeille (*Apis mellifera*) infestées par le *Varoa jacobsoni*, et qui ont donné des résultats prometteurs. Cette plante a donné un rendement en huile essentielle très important de 2,34%. Son effet toxique est testé sur les adultes d'abeille de 16 ruches alignées et réparties en 4 lots (trois par l'huile essentielle et un par l'Apiston) au champ afin d'obtenir la toxicité réelle sous les conditions naturelles du milieu. 3 doses d'huile essentielle (D1=20ml, D2=10ml et D3=5ml) diluées dans 1000ml d'huile de tournesol de table (Cevital), qui a déjà prouvé sa non toxicité sur ce parasite. De chaque dose, 5 ml sont mis dans des lanières en carton, bien égoutté et déposé à l'entrée des 3 ruches et enfin une quatrième ruche traitée par l'Apiston. L'estimation de la mortalité des acariens se fait chaque semaine et pendant un mois, à l'aide des langes graissés mises sur le plancher des ruches selon la méthode de ROBAUX (1986) et BARKANI (1985). Le taux d'infestation des 4 lots avant traitement était hétérogène (18,29% ; 14,32% ; 14,63% et 20,22%) respectivement, et a nettement diminué par le traitement D1 à une valeur variant entre 0,20% et 8,16%. L'Apiston a fait diminuer l'infestation entre 0,21% et 9,84%. L'efficacité de l'huile essentielle d'origan sur le *Varoa* est obtenue par la dose D1 après 4 applications où le taux de mortalité a atteint 98,77% avec un taux d'efficacité de 86,84%. Cette huile est une solution prometteuse pour la protection de l'abeille ainsi que les produits de la ruche.

Mots clés : *Apis mellifera*, *Varoa jacobsoni*, *Origanum vulgare*, huile essentielle; activité acaricide.



Nematicidal activity of *Piper hispidinervum* (Piperaceae) essential oil

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Piper is the largest genus of the family *Piperaceae* including about 1000 species. The leaves of some *Piper* species are aromatic or have a pungent smell due to their content in essential oils with commercial importance for the fragrance and pharmaceutical industries. *Piper hispidinervum* (pimenta-longa in Brazil), is shrub distributed throughout South America. This species is especially prominent in the state of Acre in Brazil and may extend into Amazonas. As part of our ongoing project on the valorization of Brazilian native *Piper* species, we have studied the nematicidal activity of *P. hispidinervum* essential oil and its main components against the root-knot nematode *Meloidogyne javanica*.

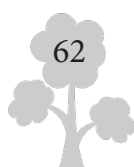
Pilot-scale steam distillations of experimentally cultivated *P. hispidinervum* fresh leaves at a pressure gradient (1, 1.5 and 2 bar) yielded three essential oils (EOs). The chemical analysis of these EOs by GC–MS showed a similar composition for the three oils, with 24 compounds, representing 98% of the total oil. Safrole was the major component (77.7 - 81.3 %) followed by terpinolene (4.6 – 8.8 %).

P. hispidinervum EOs (1 µg/µl) showed strong nematicidal in vitro effects against *M. javanica* (J2 mortality and long-term egg hatching inhibition effects). The major components (safrole and terpinolene) were not toxic to J2, but their binary combinations at different ratios induced high nematicidal activity, suggesting a synergistic interaction for these compounds in the EO.

The in vivo test of one selected *P. hispidinervum* EO (1 bar) on tomato seedlings showed strong effects on suppression of infection capacity of treated J2.

This study demonstrates for the first time the nematicidal activity of *P. hispidinervum* EO against root-knot nematodes. *P. hispidinervum* is an important plant in Brazil, which is widely exploited as a source of safrole and the nematicidal activity of *P. hispidinervum* oil could significantly increase its commercial value.

Acknowledgements: This work has been supported by grant CTQ2012-38219-C03-01. Felipe de la Peña, Ruben Muñoz and Estefania Mas are gratefully acknowledged for their technical assistance.



Evaluation de l'intérêt de l'utilisation d'huiles essentielles dans des stratégies de protection des cultures

ACCRONYME : CASDAR HE

RESUME :

Ce projet CASDAR n°1222 piloté par l'ITAB, s'inscrit dans le contexte actuel de recherche de moyens plus durables de protection des cultures, cela concerne l'agriculture conventionnelle mais également l'agriculture biologique pour lesquelles le remplacement du cuivre et la recherche d'alternatives efficaces aux produits phytosanitaires de synthèse (Plan Ecophyto, rapport Herth et feuille de route du MAAPRAT sur le Biocontrôle) constitue un important challenge. Ce projet prévoit d'évaluer l'intérêt des huiles essentielles dans des stratégies de protection des cultures sur les principales maladies des cultures (mildious de la pomme de terre, de la vigne et de la salade, tavelure du pommier) en s'appuyant à la fois sur les connaissances empiriques existantes et sur des recherches scientifiques en laboratoire (in vitro et in vivo) et au champ.

MOTS CLES : (5 au maximum)

Formulation, Stratégie, Alternatives, Fongicides, Evaluation

OBJECTIFS ET DESCRIPTION DU PROJET :

Le projet CASDAR Huiles Essentielles démarré en 2013, devra démontrer l'intérêt et l'efficacité de l'utilisation des huiles essentielles dans le cadre de la protection des cultures, pour fin 2015.

Il a pour objectif l'évaluation de 7 huiles essentielles : Eucalyptus – *Eucalyptus citriodora* ; Girofle – *Eugenia caryophyllus* ; Tea-tree – *Melaleuca alternifolia* ; Thym à thymol – *Thymus vulgaris* ; Origan sauvage – *Origanum compactum* ; Sarriette des montagnes – *Satureja montana* et Menthe verte – *Mentha spicata* afin de les intégrer dans des stratégies de protection des cultures. Les maladies ciblées sont : les mildious de la pomme de terre – (*Phytophthora infestans*), de la vigne (*Plasmopara viticola*), de la salade (*Bremia lactucae*) et la tavelure du pommier (*Venturia inaequalis*).

Ce projet s'appuie sur une approche pyramidale à plusieurs niveaux :

Niveau 1 : Au laboratoire, cette étape consiste à tester l'efficacité des huiles prises individuellement puis en mélanges sur leur capacité à enrayer la croissance mycellienne et de sporulation des champignons cultivable en culture pure. En parallèle, des tests de miscibilité avec des adjuvants de type huileux ou alcooliques seront effectués afin de proposer une formulation basique utilisable pour les essais de terrain

Niveau 2 : En milieu contrôlé, des essais seront menés sur des plantes en pots afin de définir l'efficacité des huiles et leur concentration d'utilisation optimale

Niveau 3 : En plein champ, des essais s'appuyant sur les deux premiers niveaux devront permettre de valider des itinéraires techniques incluant les huiles essentielles pour la protection des cultures.

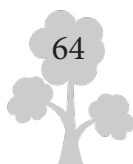
Dans ce projet, est également inclus une phase d'enquête auprès des agriculteurs utilisateurs d'HE afin de mieux connaître les pratiques empiriques de terrain.

LES PARTENAIRES :

- ADABIO
- ARVALIS
- CA 71
- CA 82
- CRAN
- FREDON Nord Pas-de-Calais
- GRAB
- IFPC
- IFV
- ISA de Lille
- ITAB
- SERAIL
- SupAgro Montpellier

Présentation :

Le poster s'appuiera sur la démarche en entonnoir (*in vitro* puis au champ), présentera les grands axes de travail et les résultats attendus.



Lutte contre *Bremia lactucae*

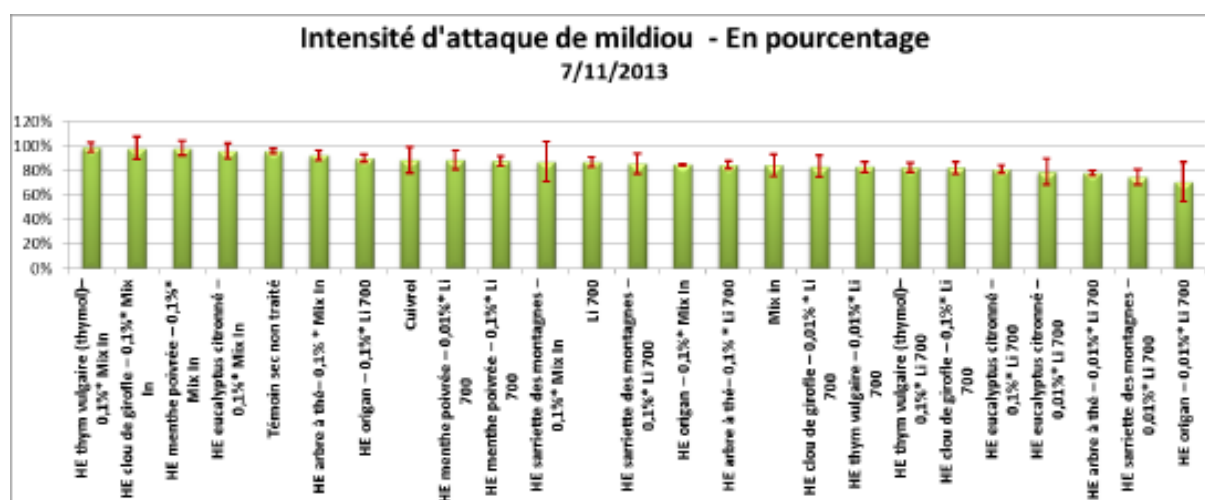
Evaluation de préparation d'huiles essentielles en conditions contrôlées – CASDAR HE

Le but de cet essai est d'évaluer en conditions contrôlées, sur jeunes plantes de laitues, des préparations à base d'huiles essentielles contre le mildiou de la laitue, *Bremia lactucae*, bioagresseur d'importance économique majeur sur cette culture. Au total, sept huiles essentielles (HE) associées à deux co-formulants ont été évaluées et comparées entre elles ainsi qu'à un témoin non traité. Les co-formulants qui ont été retenus étaient le LI 700 (lécithine de soja à 0,5%) et le Mix In, communément appelé « huile végétale », il s'agit d'un ester méthylique de l'huile de colza. Ce dernier est un adjuvant relativement générique et présente la meilleure miscibilité moyenne avec toutes les HE* testées. Le Mix In s'emploie à 0,2%.

Deux doses d'huiles essentielles ont été testées à 0,01% et 0,1% après un premier screening en laboratoire, associées avec un des deux co-formulants (LI 700) et 0,1% pour l'autre (Mix In).

L'essai a été conduit en plateaux de mottes de laitue, *Lactuca sativa*, variété Icaro, certifiée en AB et élevés en pépinière verre. Deux contaminations par pulvérisations de spores de *Bremia lactucae* ont été réalisées, directement sur les plants et trois applications d'huiles essentielles ont été réalisées.

En termes de résultats, les symptômes de mildiou sont apparus tardivement mais leur évolution a été exponentielle en fin de cycle.



Aucune huile essentielle ne se distingue en termes d'efficacité contre le mildiou de la laitue et aucune différence n'est constatée par rapport au témoin non traité.

Toutes, aux deux doses, associées au co-formulant LI 700 ou au Mix In, ne se différencient pas du témoin non traité. Le Cuivrol ne se différencie pas du témoin non traité, ni les deux co-formulants testés seuls.

Concernant les co-formulations, il semble que le LI 700 apporte une efficacité légèrement supérieure au Mix In.

Aucun symptôme de phytotoxicité visuelle n'a été observé sur l'ensemble des modalités.

Aucune mesure de récolte n'a été réalisée du fait de l'hétérogénéité de croissance des plants (liée à la méthodologie de l'essai), sur l'ensemble des modalités.

Cet essai sera reconduit a été reconduit en 2014, sous pépinière avec une méthodologie similaire, exceptée la contamination de mildiou, réalisée à l'aide de plants inoculateurs et non par pulvérisation.

Un second essai, en plein champ, sera également mis en place à l'automne 2014 afin de vérifier les résultats obtenus précédemment.

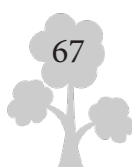
Analyse de la croissance et le pouvoir antifongique de *Gelidium sesquipedale* de la côte ouest algérienne.

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La valorisation d'une rhodophycée, *Gelidium sesquipedale*, du littoral méditerranéen algérien, exige l'aménagement de son éventuelle exploitation qui reste tributaire de la connaissance de sa biologie. C'est dans ce but, qu'un suivi mensuel de la croissance de l'algue dans son milieu naturel, a été effectué pendant un cycle annuel au niveau du site de Sidi Mejdoub (wilaya de Mostaganem) sur les côtes ouest algériennes. Cette zone est caractérisée par un substrat rocheux qui abrite une diversité benthique importante au niveau de l'étage médiolittorale. Les paramètres de croissance étudiés (Poids, longueur du thalle, Nombre totale des ramifications) ont montré des valeurs maximales en période estivale et minimales en période hivernale. L'analyse d'extrait algale récolté durant la période estivale, nous a permis de mettre en évidence une activité antifongique importante vis-à-vis la croissance d'un champignon phytopathogène *Fusarium oxysporum*. Globalement, les résultats de nos investigations font ressortir la saison estivale comme la meilleure période de récolte de *G. sesquipedale* en vue de son exploitation.

Mots clés : Rhodophytes, *Gelidium sesquipedale*, paramètres de croissance, activité anti- fongique, côte ouest Algérienne.



Action des huiles essentielles de quatre espèces de *Rutaceae* sur les paramètres biologiques de la bruche du Haricot *Acanthoscelides obtectus* Say

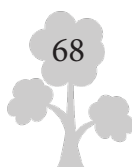
(Coleoptera : Chrysomelidae, Buchinae)

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et Goucem Khelfane Karima³

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La bruche du haricot *Acanthoscelides obtectus* Say est un insecte cosmopolite, polyvoltin, potentiellement ubiquitaire pouvant infester, outre sa plante-hôte *Phaseolus vulgaris*, d'autres légumineuses vivrières d'importance économique pour les pays en développement. C'est un ravageur potentiel qui peut détruire jusqu'à 80% de la récolte. L'utilisation de pesticides de synthèse étant de plus en plus problématique, il y a recours actuellement aux produits naturels notamment les plantes aromatiques qui recèlent un véritable arsenal insecticide ou insectifuge capable d'assurer la protection végétale. L'effet des huiles essentielles de quatre espèces végétales de ma famille des *Rutaceae* est testé par contact, inhalation et répulsion. Les résultats montrent que l'utilisation des huiles de ces Citrus dans les traitements par contact réduisent la longévité des adultes d'*A.obtectus*. L'huile du bigaradier (*C.aurantium amara*) induit une mortalité de 100% à la dose de 6µl avant 24heures, alors que les autres entraînent une mortalité de 100% à la dose de 10µl avant 12,30et 18 heures respectivement pour les huiles du citron (*Citrus limonum*), l'orange (*C.sinensis*) et le pamplemousse (*C.paradisi*), par conséquent aucune descendance n'est enregistrée à ces doses. L'utilisation des huiles de ces Citrus est avantageuse, vu qu'elle limite les pertes en poids des graines de *P.vulgaris*, ainsi que le pouvoir germinatif de celles-ci qui reste non altéré aux plus fortes doses. En ce qui concerne le test de répulsion, les huiles ont montré des effets différents à l'égard de l'insecte, il est constaté que l'huile du bigaradier est répulsive avec un taux de 70%, l'huile du citron et le pamplemousse sont modérément répulsive avec des taux de 50% et 42,5% respectivement, alors que l'huile de l'orange présente un effet très faiblement répulsif avec un taux de 17,5%. De même, les huiles essentielles ont montré des effets inhalateurs différents à l'égard des bruches ; l'huile du bigaradier manifeste un effet de fumigation très marqué même aux plus faibles doses, toutes fois les huiles du ciron, l'orange et le pamplemousse ont révélé un effet notable exclusivement aux plus fortes doses.

Mots-clés: *Acanthoscelides obtectus*, *Phaseolus vulgaris*, huiles essentielles, Citrus, lutte biologique



Fungal Solid-State Fermentation products to control plant-parasitic nematodes

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¹ *Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), AMU 13397 Marseille - France.*

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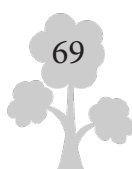
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Global agricultural losses caused by nematodes are considerable (Mateille et al., 2007). Worldwide, more than USD 125 billions/year are lost due to nematodes, mostly to Root-Knot Nematodes (RKN) (Brand 2006). The importance of these damages can be even more severe as nematodes can increase plant susceptibility to other parasites (Bridge et al, 2005).

In the Souss-Massa Draa, Morocco, RKN can cause great damages on tomato culture. The increasing interdiction of the use of disinfectants, like Methyl Bromide, and legal Pre-Harvest Intervals (PHI) prohibiting harvest after chemical application (fungicide, herbicide, insecticide) let the vegetable producers' in a situation of non-alternative issue to fight that pest and save yields and fruit caliber. Biocontrol Agents like bacterias, viruses or fungi are natural auxiliaries that can be used to fight and reduce pest populations to conserve a sustainable economic level of production (De Bach 1964, Stirling 1991). Some fungi are known to be nematophagous ; they are carnivorous fungi specialized in trapping and digesting nematodes (Cayrol et al. 1992), like *Paecilomyces lilacinus*, *Arthrobotrys oligospora*, *Verticillium chlamydosporium*, etc. Furthermore, a fungus named *Trichoderma harzianum* is able to produce some metabolites that can be used to control nematodes: chitinases, lipases, 6-pentyl- α -pyrone (Roussos, 1985, Samson et al. 1996)

Solid state fermentation is an ancestral and ecological process well-known to produce high level of biomass and fungal metabolites. It can be used to produce conidiospore and enzymes of *Trichoderma harzianum* to control RKN in Moroccan tomato fields. Indeed, chitinases, lipases and esterases can attack the nematode skin. Scale up of these productions is being done at the IMBE laboratory, and a new type of disposable bioreactor was set up and patented by IRD. It can produce a large amount of microbial spores to treat until 50 Ha of tomato crops in Morocco in 10 fermentation days.



In the IRD process, cultivation of *filamentous fungi* used as biopesticides is operated on a mix of agroindustrial byproducts (sugarcane bagasse, wheat bran, potato extract, olive pomace, shrimp waste) humidified at 75% with a conidial suspension. Culture media inoculated are introduced in bioreactors with forced humid aeration during 72 hours at 30°C. After 72 hours, aeration is operated with dry air at 25°C to apply hydric stress to maintain the sporulation phase during several days to obtain a final dry product (less than 5% humidity) containing more than 2×10^9 virulent spores by final dry fermented product.

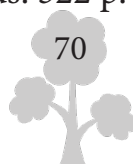
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Non-ribosomal Peptides from Entomogenous Fungi

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Abstract: Entomogenous fungi play a very important role in controlling natural population of insect pests. Many of them have been developed as myco-insecticides. In long time evolution, entomogenous fungi acquired lots of pathogenic factors against host insects. Non-ribosomal peptide (NRP) is a usual pathogenic factor of entomogenous fungi. Chemically, NRPs are the secondary metabolic compounds mainly composed of specific or modified amino acids and hydroxyl acids. They are synthesized via thio-template multienzyme mechanism of multifunctional enzyme complex system other than on ribosome. NRPs show a broad range of biological activities such as insecticide, antibiotics, antiviral, antitumor, etc. Meanwhile, researchers have been focusing the interests on the NRPs properties as immunity inhibitor, cell proliferation inhibitor, siderophore and ionophore, etc. To date, more than twenty kinds of NRPs were isolated and identified from *entomogenous fungi* genera: *Beauveria*, *Conioideocrella*, *Cordyceps*, *Culicinomyces*, *Fusarium*, *Hirsutella*, *Isaria*, *Metarhizium*, *Paecilomyces* and *Verticillium*, etc. These NRPs include bassianolides, beauvericins, beauverolides, beauveriolides, cicadapeptins, conioideocrellides, cordycommunins, cordyheptapeptides, culicinins, cyclosporins, destruxins, efrapeptins, enniatins, hirsutellides, hirsutides, isariins, isaridins, isarolides, paecilodepsipeptides, serinocyclins, etc. The structures, activity and mechanism of these NRPs were reviewed in this chapter.

Key words: entomogenous fungi; non-ribosomal peptides; structure, activity, mechanism.



1,4-disubstituted-1,2,3-triazole as a surrogate of amide bond, myths or reality? Impact of such replacement on the conformation of peptaibols

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Peptaibols are NRPS produced peptides that could elicit plant defence responses.^{1,2} Among them, Alamethicin F50/5 and Bergofungin D are two antimicrobial peptides with a helical structure.³ These two peptaibols were chosen to insert a presupposed isosteric 1,2,3-triazole motif in place of the amide bond on the Cter side of Aib residues and to assess its impact on their secondary structure.^{4,5} For this purpose an optimized solid phase synthetic strategy for preparing peptaibols of varying lengths has been developed that allows their syntheses on trityl resin using DIC/Oxyma as coupling reagents under microwave irradiation.⁶ The syntheses and results of NMR studies on six alamethicin and three bergofungin analogs will be discussed along with the preliminary biological effects of this replacement on their biological activities.

Key words: Peptaibols, 1,4-disubstituted-1,2,3 triazoles, oxyma, Alamethicin F50/5, microwave.

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L'effet des filtrats de culture de *Fusarium solani* sur la mortalité des œufs de deux espèces de Meloidogyne

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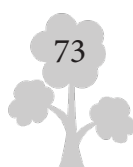
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En Algérie les nématodes à galles du genre Meloidogyne sont capables de se développer aux dépens d'un grand nombre de cultures maraîchères pratiquées en plein champ, sous serres ou même dans les oasis. Ces phytoparasites se distinguent comme étant de redoutable ennemi et constituent un facteur limitant de production. La lutte chimique est le moyen le plus employé, mais elle n'est pas en mesure de résoudre le problème. De ce fait la mise au point de stratégies de lutte biologique par l'utilisation de champignon est suggérée dans ce travail.

L'efficacité du filtrat de culture du champignon *Fusarium solani* a été testée sur les œufs de *M.incognita* et *M.arenaria*. Le filtrat est utilisé pur et dilué dans de l'eau distillée stérile ($\frac{1}{2}$, $\frac{1}{4}$ et $\frac{1}{8}$). La toxicité est estimée après 6, 24 et 48 heures d'immersion dans les différentes concentrations. Les résultats ont montré que le taux de mortalité des œufs des espèces étudiées augmente avec la concentration le temps d'immersion. Après 48 heures nous avons enregistré une mortalité de 100% pour les œufs de *M.arenaria* et 88 % pour ceux de *M.incognita*.

La toxicité du filtrat de culture *Fusarium solani* est due à la présence d'acides gras volatiles révélés par la chromatographie en phase gazeuse (CPG). Ces métabolites toxiques sont à faible poids moléculaires produits durant le développement du champignon dans le milieu liquide (Czapek.Dox). Ils sont représentés par acide formique, acide acétique et acide butyrique à des concentrations respectives de 45.10^{-2} , 5.20 et 85.10^{-2} mole/l.

Mots clés: Filtrat de culture, *Fusarium solani*, Meloidogyne, Mortalité, Œufs.



Abstract

The effect of *Fusarium solani* filtrates on the mortality of eggs of two species of Meloidogyne

In Algeria the root-knot nematodes Meloidogyne are able to develop on a great number of vegetables practiced in full field, under greenhouses or even in the oases. These plants parasitic are distinguished as being from frightening enemy and constitute a limiting factor of production. The chemical control is the most used way, but it is not able to solve the problem. This fact the development of strategies of biological fight by the use of fungus is suggested in this work.

The effectiveness of the filtrate of culture of *Fusarium solani* was tested on eggs of *M.incognita* and *M.arenaria*. The filtrate is used pure and diluted in sterile water distilled ($\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$). Toxicity is estimated after 6, 24 and 48 hours of immersion in the various concentrations. The results showed that the death rate of eggs of the species studied increases with the concentration and time of immersion. After 48 hours we recorded a mortality of 100% for eggs of *M. arenaria* and 88 % for those of *M. incognita*.

The toxicity of the filtrate of culture *Fusarium solani* is with the presence of fatty acids birds revealed by chromatography (CPG). These toxic metabolites are low-weight molecular and products during the development of fungus in the liquid medium (Czapek.Dox). They are represented by acid formic, acid acetic and acid butyric with concentration respective of 45.10^{-2} , 5, 20 and 85.10^{-2} mole/l.

Key words: Filtrate of culture, *Fusarium solani*, Meloidogyne, Mortality, Eggs,

Endophyte *Penicillium glabrum* as potencial source of biopesticides

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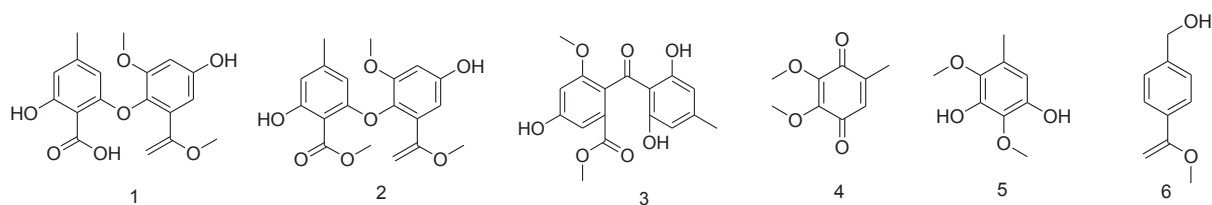
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Endophytes are microorganisms that live in the intercellular spaces of plant tissues producing bioactive substances that may be involved in the host-endophyte relationship¹. These microorganisms represent a biotechnological source of bioactive metabolites.

As part of our ongoing study of the endophyte biodiversity of the Canarian Laurel Forest, we have isolated *Penicillium glabrum* from *Persea indica*, one of the dominant Lauraceae tree species² reported as a potential source of insect-control agents³.

P. glabrum is known for the production of the bioactive metabolites asterric acid and its derivative sulochrin⁴. Asterric acid has been reported to inhibit endothelin binding *in vitro*⁵⁻⁶, while methylasterric acid and sulochrin showed anti-angiogenic activity⁷. In addition, sulochrin is an inhibitor of hepatitis C virus infection in cell culture⁸.

The endophytic isolate *P. glabrum* was fermented in liquid medium for 7 days. The organic extract showed antifeedant effects against several insect species. Furthermore, we have isolated asterric acid (1), methyl asterric acid (2) and sulochrin (3) as the major compounds, and the benzene derivatives (4), (5) and (6) in lower amounts.



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Développement d'une stratégie de lutte alternative contre *Phytophthora infestans*, pathogène responsable du mildiou de la pomme de terre

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Phytophthora infestans est l'agent pathogène responsable des symptômes de mildiou de la pomme de terre. En France, il peut entraîner la perte partielle ou totale de la production et peut également causer d'importantes pertes lors du stockage. Pour lutter efficacement contre ce pathogène, de nombreux fongicides de synthèse sont présents sur le marché mais sont souvent très polluants pour l'environnement. Dans la quête de solutions alternatives qui visent à réduire l'utilisation de produits phytosanitaires (Plan Ecophyto), la SIPRE a mis en place un programme de recherche basé sur le développement de nouvelles stratégies de lutte utilisant des microorganismes naturels antagonistes. Quatre bactéries du genre *Bacillus* ont été isolées de la phyllosphère et de la rhizosphère de plants de pomme de terre pour leur activité d'antibiose contre *Phytophthora infestans* en condition *in vitro*. Des expérimentations sous serre sont actuellement en cours pour évaluer la durée de survie de chacun des antagonistes sur feuilles afin de définir une fréquence d'apport ainsi qu'une concentration optimale des bactéries apportées seules ou en *consortium*. Un pathosystème pomme de terre/mildiou est en cours de développement pour permettre l'évaluation des différents antagonistes sur l'apparition de symptômes sur feuillage.



A new molecular tool

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A new molecular tool named qPFD® has been developed and patented by INRA for apple, tomato, grapevine, and potato crops, to validate and characterize the efficiency of candidate products as plant defense elicitors.

This tool is now proposed by VEGEPOLYS to select candidate products before field evaluation and to accelerate the development process.

The methodology is described as well as results obtained in leaf tissues of the four crop species after treatment with the same two elicitors. Interestingly, differential responses are induced according to the plant tested.

Amélioration de la croissance des plantules de piments par suite du prétraitement de ses graines par les extraits aqueux d'algues

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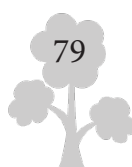
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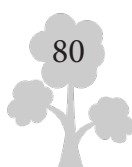
Résumé

Les extraits aqueux de thalles d'une algue rouge (*Jania rubens*) et une algue brune (*Padina pavonica*) ont été utilisés pour le prétraitement de graines de deux variétés (Baklouti et Chargui) de piment (*Capsicum annuum* L.). Les graines trempées pendant 24 heures à $28 \pm 2^\circ\text{C}$ dans les extraits d'algues (à 20, 40, 60, 80 et 100g/l) puis lavées soigneusement à l'eau distillée, séchées à proximité du poids initial puis cachetées et stockées à 5°C jusqu'à utilisation ont constitué un premier lot (GNS), un second lot correspondant à des graines qui ont été placées dans les boîtes de Pétri à germer directement après prétraitement puis lavage (GS). Les graines prétraitées ont été mises à germer puis les plantules de piment des deux variétés ont été repiquées dans des pots et irriguées tous les deux jours. Des graines non traitées ont servi de témoins. Les résultats ont montré que la croissance des plantules de la variété Baklouti ont été améliorées dans les deux cas de prétraitement (GS et GNS) par les extraits de *J. rubens*. Dans le premier cas, les stimulations des racines sont passées de 21 à 97.5% pour les concentrations allant de 40 à 100 g/l. Dans le deuxième cas de prétraitement (GNS) les meilleurs résultats ont été notés pour des concentrations inférieures à 80g/l où une stimulation moyenne de 173% a été enregistrée, au-delà les valeurs des stimulations ont été de 31 et 94%. La meilleure amélioration de la croissance des racines de la variété Chargui a été enregistrée à 80g/l avec des stimulations respectives de 37% et 16% pour les celles provenant des GS et les GNS. Le prétraitement par les extraits de *P. pavonica* a nettement amélioré la croissance des racines de la variété Baklouti, avec des stimulations d'une moyenne de 87% et 93% pour celles provenant des GS (40 et 80g/l) et des GNS (100 g/l), respectivement.



Concernant la variété Chargui et suite au prétraitement par les extraits de *P. pavonica*, une stimulation moyenne de 23% a été enregistrée pour les GS à toutes les concentrations, alors que pour celles des GNS, une inhibition de 24% a été notée pour les concentrations allant jusqu'à 80 g/l et un effet non significatif à 100 g/l. Les stimulations de la longueur des parties aériennes de Baklouti, provenant des GNS, ont varié entre 99 et 389% pour les différentes concentrations des extraits de *J. rubens*. Avec le même type de prétraitement par les extraits de *P. pavonica*, les valeurs ont varié entre 80 et 324%. Dans les deux cas, le maximum a été enregistré à 40 g/l. Pour les plantules de Chargui provenant des GS prétraitées par les extraits de *P. pavonica*, les parties aériennes ont accusé une meilleure croissance comparées aux racines, avec un comportement similaire à toutes les concentrations, la stimulation moyenne a été de 87%. Les résultats montrent que les extraits aqueux des thalles de *Jania rubens* et de *Padina pavonica* pourraient être considérés comme étant un outil efficace dans l'amélioration de l'émergence et la vigueur des plantules et pourraient être utilisés dans les prétraitements des semences et remplacer ainsi les produits chimiques redoutables.

Mots clés: Prétraitement, croissance, piment, extrait aqueux, algues.



***Trichoderma atroviride*, strain I-1237, reduces the impact of *Pythium* in carrot crop production**

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Vegetable crop production is subject to the attack of several fungal soilborne pathogens, significantly reducing yield and quality. Fungi responsible for these diseases are present in most French agricultural soils. Chemical control products against these diseases are widely available in the market, however efficient, residues on the crop and eco-toxicological concerns have led to a demand of low-risk products.

Agrauxine has developed the microbiological solution Tri-Soil® WP using as active substance *Trichoderma atroviride* I-1237. Preliminary lab tests to determine the mode of action of I-1237 showed a good potential for the biocontrol of soilborne diseases, based on the outgrowth on agar plates of the antagonist over the pathogens *Pythium* sp. and *Rhizoctonia* sp.

In the field, *Trichoderma atroviride* I-1237 proved its efficacy against *pythium* cavity spot on carrots, attaining a protection level similar to the chemical reference. On lettuce production, the antagonist was able to protect against *rhizoctonia* rot also as efficiently as the conventional reference.

On a qualitative level, field trials showed that lettuces treated with I-1237 had a higher weight at harvest than non-treated controls, similarly, roots of treated carrots were bigger, and the vitality of plants was improved.

This specific strain of *Trichoderma atroviride* demonstrated that it is a suitable alternative for conventional treatments and can therefore be part of an integrated management program of vegetable crops soilborne diseases

Elicitra is a French network

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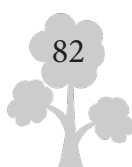
Background

Agriculture must face challenging and apparently contradictory issues: becoming both competitive and sustainable. The current reduction plan of pesticide use, occurring throughout Europe, must therefore be accompanied by the development of efficient environmentally friendly methods in crop protection. Among them, the enhancement of plant defence mechanisms by elicitor treatments is one of the most promising strategies and has become a major topic of current research.

Elicitra network

Elicitra is a French network co-animated by ARVALIS-Institut du vegetal, Vegenov and INRA. Its main mission consists in promoting plant protection by induced resistance through research, training and development. This network is dedicated to a large range of plant productions: field crops, vegetable, fruits, vine, ornamental plants and medicinal plants. It includes partners from public research, technical institutes, universities, agricultural colleges, various actors of the crop industries and competitive clusters. By bringing together various partners with different skills ranging from field to lab and from research to training, the understanding and development of this alternative approach is accelerated.

Elicitra is supported and financed by the French ministry of agriculture. It was launched in 2011 and will work until 2018.



Main Results

Network & communication

- An active community about elicitor
- A web site : www.elicitra.org (in French)
- A 2 days meeting in 2013 (150 participants)
- A scientific & technical monitoring : Elicitr'Actu (4 times a year)

Scientific & technical results

- A definition of what is an elicitor
- A data base with the main experimental results
- A guide for experimental practices

Impact on research

- Reorientation of public research priority
- A list of research priority (based on an exhaustive work about plants diseases and elicitation potential progress)
- New research projects

Next objectives

During the next 4 years, Elicitra will keep on working about sharing results, communication and research with 5 main axes: assessment of new elicitors, plant response to elicitor, application condition, elicitor in IPM and agro ecology, unintended effects.

Elicitra is an open network: contribution and exchange with elicitor actor (in France and abroad) are welcome.

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